National Institute
of
Allergy and Infectious
Diseases

Annual Report of Intramural Activities

October 1, 1992-September 30, 1993

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

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For Administrative Use

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1993 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI

00013-30 LIP
00020-18 ODIR
00027-26 LMM
00030-25 LI
00035-18 LI
00036-28 LI
00057-20 LCI
00058-19 LCI
00072-22 LPVD
00074-21 LPVD
00085-16 LPVD
00086-16 LPVD
00094-34 LPD
00097-35 LPD
00098-37 LPD
00099-23 LPD
00102-19 LPD
00108-22 LMR
00123-27 LVD
00126-20 LVD
00134-31 LI
00138-16 LIP
00144-29 LIG
00145-26 LIG
00155-18 LHD
00161-16 LPD
00162-17 LPD
00166-16 LIG
00168-16 LIG
00169-16 BRB
00170-16 LIG
00171-16 LIG
00172-15 BRB
00180-15 LIG
00190-15 LMM
00193-14 LMSF
00197-14 LPD
00208-13 LMR
00210-13 LIR
00213-13 LIR
00216-13 LICP
00224-12 LI
00226-12 LI

Z01-AI

00356-11 LCI
00358-11 LIR
00361-11 LIR
00368-11 LID
00370-11 LID
00372-11 LID
00389-09 LIG
00390-10 LIR
00394-10 LI
00403-10 LI
00415-09 LMM
00416-10 LVD
00418-10 LPVD
00425-09 LI
00427-09 LI
00430-09 LCI
00431-09 LIR
00432-09 LCI
00439-09 LPD
00445-09 LVD
00446-09 LID
00459-08 LID
00465-08 LIP
00467-08 LMM
00468-08 LPVD
00470-08 LCI
00480-08 LVP
00481-08 LHD
00483-08 LMR
00484-07 LIP
00485-07 LCMI
00486-07 LCMI
00487-07 LPD
00488-07 LVP
00492-07 LVP
00493-07 LI
00494-07 LPD
00498-07 LID
00507-07 LID
00512-06 LPD
00513-06 LCI
00514-06 LCI
00516-06 LMSF

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1993 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI

00519-06 LICP
00520-06 LI
00521-06 LHD
00522-06 BRB
00523-06 BRB
00525-06 LIG
00527-06 LMM
00528-06 LMM
00530-06 LID
00533-06 LID
00534-06 LID
00538-06 LVD
00539-06 LVD
00540-06 LVD
00541-06 LVD
00542-06 LVD
00543-06 BRB
00544-05 LIP
00545-05 LI
00547-05 LMM
00548-05 LCI
00549-05 LMSF
00552-05 LVP
00553-05 LVP
00554-05 LVP
00565-04 LI
00566-04 LI
00567-03 LICP
00568-04 LMSF
00569-04 LID
00570-04 LID
00573-04 LID
00574-04 LID
00576-04 LID
00579-04 LPD
00580-04 LPVD
00581-04 LCMI
00585-04 LIR
00587-04 LIR
00589-04 LCI
00590-04 LCI
00595-03 BRB
00596-03 LID
00600-03 LID

Z01-AI

00603-03 LID
00604-03 LID
00605-03 LID
00607-03 LCI
00608-03 LMSF
00609-03 LMSF
00611-03 LPVD
00612-03 LICP
00613-03 LCMI
00614-03 LHD
00615-03 LHD
00617-03 LCI
00619-03 LVD
00621-03 LCI
00622-02 LI
00623-02 LI
00624-02 LI
00625-02 LI
00626-02 LI
00627-02 LI
00628-02 LI
00629-02 LMR
00630-02 LI
00631-02 LICP
00632-02 LMSF
00633-02 LMSF
00634-02 LIR
00635-02 LIR
00636-02 LIR
00637-02 LID
00639-02 LID
00640-02 LID
00641-02 LID
00642-02 LID
00643-02 LID
00644-02 LHD
00645-02 LHD
00646-02 LHD
00647-02 LHD
00648-02 LHD
00649-02 LHD
00650-02 LCI
00651-02 LCI
00652-02 LVD

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1993 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI

00653-02 LVD
00654-02 LCI
00655-02 LCI
00657-02 LCI
00658-02 LVD
00659-02 LVD
00660-02 LVD
00661-02 LVD
00662-02 ODIR
00664-02 ODIR
00665-02 ODIR
00668-02 ODIR
00669-01 LMM
00670-01 LVP
00671-01 LVP
00672-01 LICP
00673-01 LPVD
00674-01 LCI
00675-01 LIR
00676-01 LIR
00677-01 LIR
00678-01 LIR
00679-01 LIR
00680-01 LCI
00681-01 LID
00682-01 LID
00683-01 LID
00684-01 LID
00685-01 LID
00686-01 LID
00687-01 LCI
00688-01 LCI
00689-01 LMM
00690-01 LVD
00691-01 LVD
00692-01 LIP
00693-01 LICP

TERMINATED PROJECTS

Z01 AI

00043-27 LCI
00045-23 LCI
00047-21 LPD
00048-21 LCI
00135-16 LIP
00154-17 LCI
00182-12 ODIR
00183-12 ODIR
00205-09 LIP
00218-11 LMM
00244-09 LPD
00255-11 LPD
00260-10 LPVD
00271-09 LCI
00275-10 LCI
00278-09 LCI
00279-11 LCI
00294-11 LVD
00295-11 LVD
00296-11 LVD
00297-11 LVD
00346-10 LID
00353-09 LMM
00397-07 LCI
00429-08 LCI
00437-06 LMM
00438-06 LMM
00441-06 LICP
00469-07 LCI
00476-06 LID
00477-06 LID
00478-05 LID
00495-06 LCI
00496-06 LCI
00499-04 LID
00501-05 LID
00502-05 LID
00518-03 LICP
00524-05 LPVD
00529-03 LCMI
00532-03 LID
00537-05 LIR
00546-02 LMM
00550-04 LPVD
00551-04 LPVD
00555-02 LID
00556-03 LID

Z01-AI

00557-03 LID
00558-03 LID
00562-03 LIR
00564-04 LVD
00575-02 LID
00577-01 LID
00578-03 LID
00582-02 LID
00583-02 LID
00584-01 LID
00586-02 LIR
00588-02 LMM
00590-03 LCI
00591-01 LCI
00592-01 LCI
00593-01 LCI
00594-02 ODIR
00597-02 LID
00599-01 LID
00606-01 LID
00610-02 LPVD
00616-02 LID
00618-01 LCI
00620-02 LVD
00663-01 ODIR
00666-01 ODIR
00667-01 ODIR

INACTIVE PROJECTS

Z01 AI

00143-23 LIG
00246-10 LPD
00308-07 LID
00451-08 LID
00476-05 LID
00477-05 LID
00500-06 LID
00531-05 LID
00556-02 LID
00571-03 LID
00572-03 LID
00575-01 LID
00598-02 LID
00601-02 LID
00602-02 LID

TRANSFERRED PROJECTS

Z01-AI

00047-21 LPD
(FROM LCI)
00108-21 LMR
(FROM LPD)
00155-16 LHD
(FROM LCI)
00208-12 LMR
(FROM LPD)
00240-11 LMR
(FROM LPD)
00241-11 LMR
(FROM LPD)
00248-11 LMR
(FROM LPD)
00481-06 LHD
(FROM LCI)
00483-07 LMR
(FROM LPD)
00521-04 LHD
(FROM LCI)
00595-02 BRB
(FROM LCI)

OFFICE OF THE DIRECTOR, DIVISION OF INTRAMURAL RESEARCH, NIAID
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SUMMARY OF PROGRAM
Laboratory and Clinical Research, NIAID
October 1, 1992 - September 30, 1993

The Division of Intramural Research (DIR), NIAID consists of 16 laboratories, a Biological Resources Branch, Administrative Management Branch and an Animal Care Branch. Four laboratories are located in Hamilton, Montana at the Rocky Mountain Laboratories (RML). The remaining facilities are concentrated at the NIH Bethesda Campus with auxiliary facilities at the Frederick Cancer Research and Development Center, Frederick, and Rockville, Maryland. The Office of the Director, DIR, (also called the Office of the Scientific Director, NIAID) is responsible for the management of the Division of Intramural Research as well as advising the Director, NIAID on scientific issues. In FY '93 the DIR, NIAID had an allocation of $123,407,000. The allocation of the budget was as follows: 34% for salaries, 42% for laboratory support, 9% for the management fund and 15% for patient costs. In FY '93 DIR employed 90 tenured scientists, 4 physicians with clinical tenure, 21 tenure-track scientists, 534 non-tenured scientists (including guest workers, special volunteers and summer students) and 415 non-scientists (technicians, administrative workers and other support staff). Included among these were individuals filling 655 FTE's.

Scientific Activities

Numerous outstanding accomplishments were made by DIR, NIAID scientists in FY '93 and are detailed in the complete Annual Report. Some of the many highlights listed in alphabetical order by topic, were as follows:

AIDS

o LIR scientists are delineating the mechanisms whereby the human immunodeficiency virus (HIV) causes a progressive destruction of the body's immune system leading to clinically apparent diseases. They have demonstrated that the lymphoid organs are a major reservoir for HIV in the body. In these sites, the virus actively replicates early in the course of infection when the patient is experiencing a period of clinical latency. These studies demonstrate that complete microbiological latency never exists during the course of HIV infection and that HIV replication and disease inexorably progresses even during a prolonged asymptomatic period. On the basis of these findings, it is important to determine the effect of anti-retroviral therapy in the lymphoid organs, which are the primary site of viral replication. In this regard, a study involving two lymph node biopsies, the first before starting the anti-retroviral therapy and the second after eight weeks of therapy, is directly addressing this issue. Furthermore, the scientists are investigating in peripheral blood and lymphoid organs the levels of expression of certain immunoregulatory factors, (cytokines) that are capable of inducing or suppressing the replication of HIV. These studies have important implications in the design of therapeutic strategies.

- LMM scientists are investigating the function of the Vpu protein, which is unique to HIV-1 and does not have a known homologue in HIV-2 or SIV. The researchers have shown in the past that this viral factor is incorporated into membranes of the host cell and enhances the release of virus particles from infected cells. They now have evidence that Vpu has a second function. Expression of Vpu in host cells causes rapid degradation of CD4, which acts as the receptor for HIV when the virus first enters a T cell. Both functions of Vpu are likely to have negative consequences for HIV-infected individuals. Enhancement of virus release leads to an enhanced progression of the disease, and the loss of the CD4 molecule in infected T lymphocytes severely interferes with the normal function of these cells in the immune system. The development of a drug to inhibit Vpu may slow the spread of HIV in the immune system and improve the efficacy of other drugs.

- LPVD scientists are studying how and why different HIV strains in AIDS patients can infect different types of cells, such as T lymphocytes, macrophages, and brain cells. By exchanging pieces of viral DNA between HIV clones capable of infecting different cell types, they have found that a small segment of the viral envelope protein has a major influence on determining the ability of an HIV strain to infect a particular cell type. These results may help explain why different AIDS patients have different clinical symptoms. They may also have some bearing on the selection of appropriate HIV strains to use in potential HIV vaccines. Recent results show that particular amino acids in the envelope V3 variable region strongly influence which cell types a particular HIV strain will infect. Surprisingly, the V3 regions of HIV strains that infect macrophages are not very variable; therefore V3 regions might be good target for an HIV vaccine.

- LIP scientists are seeking to determine the mechanisms by which retroviruses induce immunodeficiency, how this type of disorder can be prevented and how it can be treated. Use is made of a murine leukemia virus (MuLV) model system in mice, designated mouse AIDS (MAIDS), that has many similarities to the course of HIV infection in humans. Mice differ greatly in their response to this infection, some having a near normal life span with no signs of MAIDS and others dying of severe disease within months of inoculation. These differences in response to infection are determined by specific genes that regulate immune responses in normal mice to produce a balance of antibodies (humoral response) and cellular immunity. The balance between humoral and cellular immunity is controlled by a series of cytokines produced by CD4+ T cells. MAIDS- resistant mice produce cytokines that direct a strong cellular but no humoral response, while MAIDS-sensitive mice produce cytokines that drive primarily a humoral response. NIAID researchers have found that the nonprotective response of sensitive mice can be redirected to make them disease-resistant by treatment with a recently described cytokine designated interleukin-12 (IL-12). If MAIDS is an accurate model of HIV infection in humans, these results suggest it would be important when immunizing against HIV to develop methods that result in optimal cellular responses to infection. Vaccines might be engineered to include non-HIV genes that would influence the immune system towards a cellular response. Similarly, treatment of HIV-positive patients with cytokines or other drugs that would push the immune system towards a cellular response could be important in the elimination of virus-infected cells.

o LI researchers are seeking to develop methods for improving the immune response to HIV-1 so that vaccines to prevent infection and treating already infected people can be developed. Using mice, the scientists have identified several regions in the proteins (gp120, pol) of HIV-1 that bind to major histocompatibility complex (MHC)-encoded class I or class II molecules. These peptide-MHC molecule complexes are the key to immune recognition by T lymphocytes. The regions identified using mice have been also shown to be the targets for T cell recognition by human T cells. Because HIV-1 changes the structure of its genes and proteins very often, it can escape from detection by immune T cells that have learned to recognize a different form of the virus. It is therefore important to develop methods to induce very broad or cross-reactive immune responses that make it hard for such changes in virus structure to allow escape from immune destruction. By studying in detail the effect of altering the sequence of the HIV peptide antigens, the scientists have been able to induce very broadly specific killer T cells in mice. This method may be useful in designing a vaccine against HIV-1 that would work in many people and against many strains of the virus. The researchers have also identified regions of the virus that stimulate different types of immune T cell responses. This knowledge can be used to design vaccines that augment the helpful responses and avoid, or even shut off, the harmful ones. Finally, the scientists found that varying the native structure of an HIV antigen increased its ability to stimulate T cells many fold. This knowledge should help to improve the efficacy of vaccine components so that vaccines can be made more potent at less cost.

o Simian immunodeficiency virus (SIV)- infection of macaque monkeys is a relevant animal model to test potential vaccine strategies for AIDS. LID researchers have used this animal model to study various vaccines. Studies were initiated with a killed whole virus vaccine. This vaccine protected immunized animals from infection with cell-free virus but not from SIV-infected cells. Although immunized animals that were challenged with infected cells became infected, their survival was greatly prolonged as compared to unvaccinated monkeys. Another vaccine that is currently being tested involves the use of vaccinia virus vectors to express viral proteins of SIV. These studies use a vaccinia virus that has been modified to express large amounts of the SIV envelope protein and also altered so as not to induce a lesion at the site of vaccination.

o The SCID-hu-PBL mouse project is an attempt to develop an appropriate animal model for HIV-1 infection by replenishing the immune systems of severe combined immunodeficient (SCID) mice with human peripheral blood lymphocytes (hu-PBLD). In that the resulting immune system contains elements from both mouse and human sources, this model bridges the gap between the testing of new drugs solely in test-tube experiments and the evaluation of such agents in clinical trials in humans. In work on this model so far, LIR researchers have been able to successfully transfer human lymphocytes into the mouse host by intraperitoneal injection which remain viable for several weeks or more. They have also demonstrated that these lymphocytes can be infected after transfer by inoculation of the animals with HIV-1. Pretreatment of the animals with certain antiviral drugs, such as zidovudine, provides relative protection against this infection. Using this model, the researchers have demonstrated that a new anti-retroviral drug called FddA has activity that is consistently superior to zidovudine in terms of its ability to prevent new infection or to suppress infection that is already present. In contrast, transfer of lymphocytes from a healthy donor previously immunized against the major envelope protein of HIV-1 did not result in enhanced protection against viral infection in this model.

o LIR scientists are interested in identifying those aspects of the human immune system that serve to keep the human immunodeficiency virus (HIV) in check. A series of clinical trials have been initiated or are continuing. A protocol that attempts to reconstitute the immune system in infected individuals utilizes transfer of blood immune cells from an HIV negative identical twin to the infected sibling. So far, these studies show that temporary improvement in the immune system results from the cell transfers, but the cost may be an increase in the amount of HIV virus that actively divides in the body. In a separate study, blood cells were removed from the body of an HIV-infected patient. Cells were programmed to recognize and kill HIV-infected cells, then returned to the same patient in large numbers. The one patient who participated tolerated receiving the cells on multiple occasions but showed no improvement in his condition. Protocols studying the effects of interleukin-2 (IL-2) in combination with other anti-HIV drugs are in progress. IL-2 is a natural substance that can activate the immune system and increase the numbers of CD4 cells, the cells particularly damaged by HIV. The researchers have defined the maximal dose of IL-2 that can safely be administered with zidovudine and are now studying the long term effects of IL-2 on HIV disease. The investigators have completed a study of N-acetylcysteine in HIV. This drug is believed to prevent oxidative damage to cells and to inhibit HIV from growing. They found that the drug is poorly absorbed from the intestinal tract and lacks activity against HIV at the doses studied.

ASTHMA/ALLERGY

o LCI scientists have found that allergen exposure induces several immunologic responses in the human nasal mucosa, including activation of lymphocytes (increased expression of the interleukin-2 receptor), and production of lymphokines. Using a newly developed technique for measuring cytokine production by nasal mucosal cells, will determine the role of cytokines in the airway hyper-reactivity found in asthma and rhinitis.

o LCI scientists have found that the neuropeptide endothelin-1 (ET-1) is located within glands in the nasal mucosa and is included in nasal secretions. The researchers have also identified the muscarinic M3 receptor as being the type of receptor that is primarily involved in the regulation of nasal glandular secretion by acetylcholine. These findings will facilitate pharmacologic approaches at modulating mucus secretion.

- When T cells respond to antigen, they develop into cells that secrete a potent set of proteins called lymphokines. Of these, two are particularly important interferon gamma (IFN-γ) and interleukin-4 (IL-4). In general, T cell responses are dominated by cells that produce IFN-γ but not IL-4 or cells that produce IL-4 but not IFN-γ. Furthermore, immune responses dominated by IFN-γ production are very effective against infectious agents that establish themselves within cells, which is thought to be the case for M. tuberculosis. By contrast, immune responses dominated by IL-4 production are effective against certain types of extracellular parasites but can also lead to allergic conditions. LI scientists have studied what determines the decision of the T cell to become an IFN-γ or IL-4-producer. They found that IL-4 promotes the differentiation of naive T cells into IL-4 producers and inhibits T cells from becoming IFN-γ-producers. Some T cells become IFN-γ-producers in the absence of IL-4, but this process is enhanced by the addition of the recently described cytokine, interleukin-12 (IL-12). This work suggests approaches for the improvement of vaccine strategies. Thus, one might be able to bias an immune response resulting from a vaccination so that it was effective against infectious agents that establish themselves within cells by the administration of IL-12 and anti-IL-4 at the time of immunization. In addition, it might be possible to interrupt the production of IL-4 in patients with allergic diseases.

- The high affinity receptor for immunoglobulin E (FcεRI) and its ligand, the IgE antibody molecule, are involved in the allergic reaction. Allergy patients produce IgE directed against innocuous antigens (allergens), such as pollen. These antibodies bind with high affinity FcεRI on mast cells. When receptor-bound IgE is activated by allergens, a mast cell releases mediators, such as histamine. These mediators cause the symptoms of allergy, ranging from hay fever to asthma and life-threatening anaphylactic shock. NIAID scientists have produced mice in which one subunit of the FcεRI receptor has been inactivated. The mice produce a normal number of mast cells, but the cells do not express the FcεRI receptor on their surfaces. Unlike control mice, these mice are unable to enter into anaphylactic shock after antigenic challenge. These results demonstrate that by targeting FcεRI, it may be possible to cure allergic reactions.

- Allergen binds to specific antibodies (IgE) attached to FcεRI receptors expressed on various cells of the immune systems. This allergen-antibody-receptor connection triggers the cell to release the mediators of allergic reactions. In addition, to its role in allergy the FcεRI receptor may play a part in the general immune defense system. NIAID scientists have found that the FcεRI receptor is involved in eosinophil-mediated cytotoxicity against parasites. They have also detected FcεRI on the surface of Langerhans cells in human skin, and they are attempting to determine the possible role of this receptor in atopic dermatitis. Finally, FcεRI is expressed on the surface of monocytes of allergic individuals, although its role on these cells is not yet understood.

- LCI scientists have shown that anti-idiotype antibodies can replace crude food extracts to improved the reliability of diagnostic tests for food allergy. They have also identified the major protein in shrimp that causes food allergy, which should result in improved diagnosis and treatment for shrimp allergy.

CHRONIC AND PERSISTENT INFECTION

- Hepatitis C virus (HCV) is an important human virus that is the cause of the vast majority of transfusion related non-A, non-B hepatitis illnesses in the United States. The virus has also been linked to the development of liver cancer in many parts of the world. LID scientists have demonstrated that HCV is present in the blood of many patients with liver cancer. This finding strengthens the proposal that HCV is responsible for causing a proportion of liver cancer. The finding of variability within the HCV genome, especially in the genes encoding the envelope proteins, suggests that there may be heterogeneity of viral envelope proteins similar to that seen in human immunodeficiency viruses. Such a finding suggests that it may be difficult to develop an effective vaccine against HCV. The researchers determined the nucleotide sequence of one of the envelope proteins of HCV in many of the strains collected worldwide. Seven new genetic types of HCV were identified, at least some of which may represent unique serotypes. This finding has important implications in vaccine development.

- Epstein-Barr virus (EBV) is able to immortalize B lymphocytes in cell culture and is associated with certain human B lymphocyte tumors. LCI scientists have found that inoculation of EBV-immortalized B lymphocytes into severe combined immunodeficiency (SCID) mice results in EBV-containing tumors. EBV nuclear protein 2 (EBNA-2) is one of the viral proteins required for immortalization of B lymphocytes. Inoculation of SCID mice with cell lines that had different mutations in their EBV EBNA-2 gene showed that the time to formation of tumors for cells containing a given EBV mutant correlated with the ability of the mutant to immortalize B lymphocytes in cell culture. Since these findings demonstrate that the EBV EBNA-2 protein is critical for development of EBV-induced B lymphocyte tumors in animals, these studies indicate that EBNA-2 may be important in the development certain human tumors. Therefore, these studies suggest that EBV EBNA-2 may be a target for immunotherapy of certain human tumors associated with EBV.

- LCI scientists are studying how the herpes simplex virus and the varicella-zoster virus cause disease and persist in man. Each of these are herpes viruses that persist in the body for life and periodically cause recurrent infections. Herpes simplex viruses infections involve the lips, eyes and genitals. The first infection with varicella-zoster virus is chicken pox and the recurrent infection is known as shingles or zoster. Over the past decade, the researchers have developed effective antiviral treatments for these infections. They are currently studying a potent new drug for zoster know as BVaraU. This new drug is much more potent than the drug presently being used and can reduce zoster infection sufficiently to prevent its painful consequences. The investigators have also identified specific herpes virus genes that are important in stimulating the growth of the virus and other genes that are active only while the virus is in its dormant, or latent, state in people. They have also developed breeds of mice whose cells carry and express this herpes latency gene.

DIGESTIVE/ENTERIC DISEASES

o Investigators have initiated a new approach to the identification of rotavirus RNA sequences and proteins involved in viral gene expression, replication, and virion morphogenesis. They developed a simplified system in which cDNA is used to encode synthetic analogs of a rotavirus RNA. The cDNA-encoded chimeric RNAs could be rendered biologically active when introduced into tissue culture cells and complemented by proteins supplied by super-infecting rotavirus. This provides a system that should facilitate the investigation of cis-acting RNA sequences involved in RNA transcription, replication, and nucleocapsidation. This approach will be developed further to provide a system for identifying and characterizing rotavirus proteins involved in key activities. Ultimately, this approach might also be used to "rescue" synthetic cDNA-encoded rotavirus gene segments by incorporating them into infectious virus. The capability of introducing synthetic RNAs into infectious viruses represents an important line of investigation with regard to the development of live attenuated rotavirus strains for vaccine purposes.

o Inflammatory bowel disease (IBD) is a relatively common disorder that leads to severe gastrointestinal symptoms, poor growth in children, and tumors. LCI researchers have discovered that IBD patients have T cells expressing a significant decrease in the quantity of a type of T cell antigen receptor designated TCR-$V\beta_2$. This suggests that a particular antigen or superantigen is involved in causing inflammatory bowel disease.

GENE TRANSFER AND GENETIC DISEASES

o LVD scientists are substituting amino acids in a critical region of the vaccinia virus DNA polymerase to determine what structure must be maintained for the enzyme to function. The results indicate that the site requires a large amino acid that is either positively charged or neutral. Information concerning the structure of a protein can be applied to developing a drug or antibody that can target a key region in that protein and would maintain its effectiveness despite mutations.

o LHD investigators have developed two in vitro model systems to demonstrate the feasibility of genetic correction of chronic granulomatous disease (CGD). The first system uses Epstein-Barr virus transformed B-lymphocyte cell lines (EBV-BL). A replication-defective retrovirus vector, MFG, was used to transduce genes for proteins missing in different forms of CGD into EBV-BL to correct the CGD defect. The second system involves transduction of marrow-like myeloid progenitor cells from CGD patients with the MFG retrovirus containing the normal version of the specific CGD gene.

o In a critical preliminary step in the establishment of gene therapy for CGD, LHD scientists have succeeded in restoring defective enzyme production in stem cells from patients with three of four types of CGD.

o LHD scientists have identified several members of a single family with selective susceptibility to disseminated atypical mycobacterial infections. These individuals manifest a decreased ability in producing interferon gamma and their infections respond to treatment with exogenous interferon gamma. These findings may be important in the development of new techniques for treating tuberculosis.

o LMM researchers are studying the biochemical mechanisms of infectious HIV particle formation, both in terms of the HIV proteins involved and the packaging of the HIV RNA. Many of the mature internal structural proteins of HIV are derived from the proteolytic processing of two precursor proteins, GAG, and GAG-POL. The scientists have found that the self-assembly of GAG protein lays the skeletal framework of the immature particle. Coexpression of the GAG-POL fusion protein facilitates the maturation of the particle. Particle maturation is accompanied by substantial GAG processing and inclusion of viral RNA. The investigators developed a novel gene transfer and expression system to over produce the HIV GAG and POL proteins and selected HIV RNAs. HIV particles were isolated from the culture fluid and examined for RNA content. The scientists determined the minimal RNA sequence required for packaging. The cooperative interactions of RNA packaging and particle assembly are attractive targets for drug development.

o HIV-1 infects a subset of white blood cells that express a receptor for the virus known as the CD4 receptor. Once infected, these white blood cells can actively produce HIV-1 or they might remain dormant for unknown periods of time. Ultimately the infected cells are killed by unknown mechanisms. LIR scientist are seeking to engineer these white blood cells with anti-HIV-1 genes that turn off viral production within the infected cell itself or that encode a protein that is secreted and protects other cells from infection. The researchers have developed laboratory model systems in which genetically modified cells partially protected themselves and other cells against productive HIV infection. Experiments have been performed to introduce anti-HIV genes into white blood cells from HIV-infected donors. The investigators are continuing these experiments to develop gene therapy for AIDS patients.

IMMUNOLOGY

- Scientists have shown that isotype switching -- the process by which B cells transform from IgM cells to IgG or IgA cells--is preceded by a molecular mechanism marked by splicing of bits of mRNA from one end of the Ig gene (the VJD end) to the other (the VDJ end). The function of such "transpliced" mRNA is unknown as yet. However, it may play a role in forming a template for switch rearrangement, which is a later process by which the Ig gene links together the VDJ gene with C region DNA of the selected Ig class. These data may help lead to an understanding of the B cell defects in various immunodeficiencies, including IgA deficiency.

- LI researchers discovered that interleukin-10 (IL-10), which is one of the soluble messenger molecules called cytokines, inhibits the activation of T lymphocytes (T cells), not by acting directly on the T cell but by acting on an accessory cell called the macrophage. IL-10 exerts its inhibitory effects by preventing the macrophage from delivering a costimulatory signal required for T cell activation. Activation of T lymphocytes requires two stimuli. One is the specific antigen recognized by the T cell receptor and the other may be a membrane molecule expressed on a non-T cell, such as a macrophage. The scientists have shown that the specific costimulatory signal inhibited by IL-10 is an expression of the membrane molecule B7 on the macrophage surface. B7 functions by interacting with another membrane molecule on the T cell--the CD28 antigen. The requirement of the T cell for costimulation is very important because in the absence of costimulation, activation of the T cell frequently results in inhibition of T cell function. Future studies may yield important insights as to how one could suppress an inappropriate ongoing immune response in man, such as in autoimmune or allergic diseases.

- LI scientists are examining the structure and function of special proteins termed major histocompatibility complex (MHC) molecules, which are essential for antigens to be recognized by T cells. They have established that the two main types of MHC molecules, termed class I and class II, are specialized for binding to small peptide fragments of proteins located in different parts of the cell. Class I molecules are especially good at detecting antigens made by the cell, such as viral proteins or mutant proteins in a tumor cells, whereas class II molecules are better at detecting material outside of the cell, such as toxins, material shed from growing bacteria, or debris from dead or dying organisms. The researchers have discovered how the MHC molecules capture peptide antigen and use the peptide to help them fold-up into a shape that lets them move from inside the cell to the surface where a T cell can see the peptide-MHC complex. Findings from these studies provide a rational basis for vaccine antigen delivery that allows focusing the immune response on the cell-mediated effector limb (for example, CD8+ cytotoxic or killer cells that are now believed to play a critical role in resisting HIV infection) vs. the antibody producing limb (which plays an especially important role in other viral diseases such as influenza or respiratory syncytial virus). The knowledge gained by these studies has already been used by others to develop several novel means of vaccine antigen delivery to elicit CD8 cytotoxic cell responses.

o The very first step in most immune responses is the presentation of antigen by antigen-presenting cells (APC) to helper T lymphocytes (CD4+ T cells), which control the generation of cellular immune responses. Helper T cells recognize a cell surface complex of a fragment derived from the foreign protein (antigen) bound to a class II major histocompatibility molecule (MHC) of the APC. LIG scientist have found that influenza virus proteins from both outside and inside the APC can be presented efficiently to helper T cells. It was possible to distinguish at least two processing pathways for antigen delivered from outside the APC, as well as two processing pathways for antigen synthesized within the APC. Thus, the potential sources of antigen that can be recognized in the crucial initial step of immune responses are much larger than previously anticipated. The researchers also demonstrated that a very large number of class II MHC molecules reach processing compartments by rapid internalization from the cell surface of the APC, rather than by a direct intracellular route. This transport pathway may represent an important mechanism for loading class II molecules with antigenic fragments from several intracellular compartments, thus extending the range of antigens that can be recognized by CD4+ T cells. The discovery of multiple pathways through which APC can process and present antigens to CD4+ T cells has important implications in several areas relevant to many diseases, including viral infections and autoimmunity. The new intracellular pathways of antigen presentation discovered in this study provide novel approaches in vaccine design applicable to many viruses, including HIV.

o LCI scientists are studying several aspects of B lymphocyte differentiation. They have shown that there is a difference in the regulation of the germline transcript (GLT) of the immunoglobulin (Ig) genes in the first and second gene duplication units. The former requires the presence of a mitogenic (proliferative) influence plus a cytokine, whereas the latter requires only a cytokine. This indicates that early events of isotype switching are regulated at the level of Ig genes sets as well as individual Ig genes. In addition, they suggest that it may be possible to regulate B cell production of certain Ig classes independent of other classes. The independent regulation of Ig genes in the two gene duplication units helps explain the pattern of Ig subclass deficiencies in various forms of immunodeficiency.

o LCI scientists are defining some of the molecular events that occur during "isotype switching," which is the process by which B cells transform from IgM cells to IgG or IgA cells. They have shown that isotype switching is preceded by a molecular mechanism marked by the splicing of bits of mRNA from one end of the Ig gene (the VJD end) to another end of the VDJ gene, the I and C ends. The function of such "transspliced" mRNA is unknown as yet. However, it may play a role in forming a template for switch rearrangement, which is a later process by which the Ig gene links together the VDJ gene with C region DNA of the selected Ig class. These data may help lead to an understanding of the B cell defects in various immunodeficiencies, including IgA deficiency. In addition, they will ultimately help in shaping antibody responses in order to produce better vaccines.

LYME DISEASE

- Researchers found that antibiotic coumermycin A_1 inhibits the growth of Borrelia burgdorferi, which causes Lyme disease. The drug inhibits DNA gyrase, an enzyme that catalyzes supercoiling of DNA and is required for bacterial DNA replication. Results suggest that B. burgdorferi has a DNA gyrase and that this enzyme's activity is required for growth. Furthermore, structural analogs of coumermycin may be considered as treatments for Lyme disease.

- Scientists have discovered that Borrelia burgdorferi, the spirochete that causes Lyme disease, sheds membranous material from its surface during periods of growth. Detection of this extracellular material may prove to be a useful test for diagnosing active infection or monitoring treatment effectiveness. The scientists also discovered that these extracellular components are involved in the packaging and protection of intact DNA molecules and may provide a unique mode of genetic exchange. In addition, these components interact with IgM molecules in a unique way, perhaps to escape immune surveillance. They also possess potent, non-specific mitogenic activity which may cause an inappropriate stimulation of the immune system, thus triggering autoimmune disease components.

- Scientists cloned and expressed a 39 kilodalton protein of the Lyme disease spirochete that is highly reactive with antibodies in the serum of humans with Lyme disease. This antigen has been developed into a new serological test kit for confirming Lyme disease. This kit has been approved by the FDA and is now being sold in North America and Europe. The gene for the P39 antigen was sequenced. To date, 130 isolates of Lyme disease spirochetes have been examined: all have the P39 gene and all but one of the isolates express this protein.

TROPICAL DISEASES

- LPD scientists have been studying how a cytokines regulate disease and cellular immunity against the infectious agents that induce granulomatous inflammation, such as schistosomes. They have discovered that endothelial cells, which line blood vessels, can be activated by cytokines to kill schistosomes by secreting nitrites on them. The researchers are also studying how the cytokines IFN-γ and IL-12 can suppress the pathology induced by schistosome eggs in host tissues. They are currently studying the possible uses of IL-12 in immunotherapy of schistosomiasis. The investigators are also comparing their findings on cytokine expression in schistosome-infected mice with actual patients harboring the disease. White blood cells were purified from the blood of Brazilian patients and stimulated with antigens from schistosome worms and eggs. The cells from the patients responded by producing cytokines of the same type (Th2) as those previously found in mice.

o LMR researchers have developed four auxotrophic lines of malaria parasites. Auxotrophic lines require supplemental nutrients to grow either in culture or in their natural hosts. The malaria auxotrophs require P-aminobenzoic acid (PABA). The development of malaria auxotrophs should facilitate the study of these parasites, much as the development of bacterial auxotrophs did for the study of bacteria. The scientists have also isolated a new class of drug-resistant mutants of P. berghei. One set of mutants is resistant to pyrimethamine, while another is resistant to 5-fluoroorotic acid (5-FOA), drugs which interfere with de novo pyrimidine synthesis. A mechanism whereby Plasmodium malance may become resistant to pyrimethamine and 5-FOA is by elimination of pyrimidine synthesis and reliance on a salvage pathway. The drug-resistant mutants now salvage pyrimidines, a pathway not previously detectable in parasites. Studies are currently underway to define the mutations at the molecular level.

o Most malaria control strategies have been based on control of the mosquito. In sub-Saharan Africa, Anopheles gambiae is the mosquito species most responsible for transmission of the malaria parasite. LMR scientists are seeking to replace disease-carrying mosquitoes with mosquitoes of the same species who are unable to transmit malaria. To accomplish this, they are presently developing methods for applying the tools of molecular genetics to determine the population structure of the African mosquito vectors. They are also identifying and characterizing mechanisms that render mosquitoes unable to carry the malaria parasite. Finally, the researchers are characterizing the microbial components of the diet of the larval stages of An. gambiae in Africa to identify organisms that might serve as carriers of genes coding for the production of biologically active insecticides for use in larval control programs. Malaria prevention through modification of the capacity of the mosquito vector to transmit the parasite presents an attractive, first line defense against infection.

o LMR scientists are developing a malaria transmission-blocking vaccine, which is designed to prevent the transmission of the malaria parasite by blocking its development within the mosquito vector. They have been identifying molecules present in the parasite that when given in a vaccine will make malaria-infected humans incapable of transmitting malaria parasites. To date, they have identified six molecules (Pfs25, Pfs28, Pfs40, Pfs230, a parasite-produced chitinase, and a mosquito-produced protease) that are potential transmission-blocking vaccine candidates. The genetic material responsible for making four of these molecules (Pfs25, Pfs28, Pfs40 and Pfs23) has now been isolated and has been used to produce model vaccines. One of these model vaccines, Pfs25, has been found to be highly effective in producing transmission-blocking activity in mice and monkeys and will be tested in human trials in the near future. The researchers plan to develop all six of these molecules into potential transmission-blocking vaccines.

o LPD scientists have identified a number of proteins that are unique to the Leishmania parasite and that are critical for their survival. These proteins may be useful for diagnosis and may be suitable targets for destroying the parasites. The researchers have identified, cloned and sequenced a portion of the gene for 3'-nucleotidase, an essential and unique surface membrane enzyme. In addition, significant progress has been made toward obtaining the complete gene sequence for another essential leishmanial enzyme, the secretory acid phosphatase (SacP). The amastigote (Am-) form of Leishmania, which is the form that infects the macrophages of hosts, was shown to express a unique surface membrane protease (an enzyme that degrades proteins) and a unique form of SacP. Both of these were not found on the promastigote, which is the form of Leishmania that is carried in the insect vector. The alpha-tubulin (α-T) gene and a stress-inducible protein gene (STI1) were identified, cloned and sequenced from Am-. Sera from patients with visceral leishmaniasis from India but not Sudan or Brazil were found to react strongly against alpha-tubulin. Conversely, sera from visceral leishmaniasis patients from Brazil and Sudan but not India reacted strongly against the STI1 gene product. These results suggest that these Am- gene products might be useful as diagnostic reagents.
The researchers have also shown that the leishmanial SAcP appears to complex with host major histocompatibility complex (MHC) II receptors within infected bone marrow macrophages, effectively reducing their ability to present antigen to relevant T lymphocytes.

o LPD scientists are studying the differing immune reactions of individuals with filarial infections, such as lymphatic filariasis and onchocerciasis. They have found that those individuals who develop disease after infection mount a type of immune response termed Th1, whereas those who do not manifest disease despite infection have a different type of immune response, termed Th2. A Th2 response appears to be the result of the production of the anti-proliferative cytokines interleukin 10 (IL-10) and transforming growth factor-beta (TGF-β). The major source of IL-10 appears to be monocytes, although both CD4+ and CD8+ T lymphocytes are also capable of making this cytokine. Understanding the mechanisms by which some individuals are able to avoid disease despite being infected may lead to a treatment that can make all individuals immune to filarial diseases. The researchers also examined the possibility that there is a genetic predisposition to a given immune response. Novel methods of examining the major histocompatibility complex (MHC) class II genetic diversity were developed and utilized in the fine mapping of the DQ locus in patients with varying forms of filarial infection. While the data are preliminary, there appears to be a relationship between the presence of DQA301 and susceptibility to developing lymphatic obstruction.

VACCINES

- LI researchers are investigating novel approaches to vaccine development. They have chemically synthesized portions of a coat protein of HIV (gp120) from several strains and lined these moieties to cell wall material of the bacteria Brucella abortus, which provides carrier and adjuvant functions. Mice have been immunized with these materials (conjugate vaccines). The inhibition of live virus replication and the immune responses are being studied. The investigators are also creating vaccine constructs by linking antigens to soluble high molecular weight polymers. Using these vaccine constructs in animal models has shown excellent responses to diphtheria, tetanus, and pertussis toxoid challenges. Progress has also been made in developing a peptide-based malaria transmission blocking vaccine. The scientists are also developing drugs that incorporate synthetic peptides representing segments of intercellular matrix proteins. Studies so far suggest that some of these materials can block metastases of certain tumors. Inhibition of metastasis will allow time for the immune system (appropriately stimulated by other agents) to destroy established tumor tissue.

- LID scientists have shown that a virulent human rotavirus can induce diarrheal illness in a susceptible chimpanzee. They have also evaluated the pathogenicity of a cold-adapted rotavirus mutant in a single chimpanzee to determine if such a candidate vaccine strain is suitably attenuated. The chimpanzee did not develop illness or shed virus. This new candidate vaccine may prove to be more effective than current candidate vaccines and thus significantly reduce the morbidity and mortality associated with rotavirus gastroenteritis. Such a vaccine would have a profound effect on the health of infants and young children.

- The bacterium Chlamydia trachomatis is the most common agent of sexually transmitted disease in the United States, and infections can result in pelvic inflammatory disease and infertility in women. Designing a chlamydial vaccine is difficult because certain chlamydial components induce immune responses that actually contribute to disease. Furthermore, an effective chlamydial vaccine specifically needs to induce immunity at mucosal surfaces, such as the vagina. For the development of a chlamydial vaccine, LICP scientists are focusing on small regions of proteins called epitopes that are located on the surface of the chlamydial major outer membrane protein. Antibodies that bind to these epitopes can prevent the chlamydia from infecting host cells and thus prevent disease. The researchers have developed two systems for the delivery of these epitopes. One involves attenuated polioviruses in which the epitopes have been expressed on their surfaces, and in the other system, the epitopes have been genetically fused to the heat labile toxin (LT) of the bacteria Escherichia coli. Both systems are safe and effective in stimulating mucosal immune responses and have shown promise for use as chlamydial vaccines.

- Genital Herpes is a very common sexually transmitted infection. Over 20 percent of adult Americans are infected, with a slightly higher rate in women than men. LCI scientists have conducted the first human studies of a vaccine consisting of a genetically engineered protein derived from the Herpes Simplex Virus Type 2 that causes genital herpes. The initial studies have proved that the virus protein is safe and induces substantial immunity to the virus. Within the past year, 98 individuals known to have genital herpes were vaccinated. Vaccination boosted immunity to the virus, and this was associated with a 1/4 to 1/3 lower rate of recurrent infections than was seen in the subjects who were given placebo injections. These first studies involved a vaccine with just one protein known as gD2 and a common but relatively weak immune stimulant aid to vaccines know as alum. The researchers are currently investigating the benefits of adding a second virus protein called gD3 and a more potent immune stimulating adjuvant known as MF59. This is the first study in which a vaccine was able to successfully treat a chronic infection. This work has implications for the development of therapeutic vaccines in general and for the treatment of genital herpes in particular.

Administrative Activities

During the past year the DIR instituted a number of important administrative changes:

Tenure Track Policy: In response to a long-standing need to clarify and streamline the process by which the DIR recruits and retains outstanding senior staff, a new Tenure Track Policy was devised by the DIR, which was subsequently adapted, with modifications, by NIH as a whole. The salient feature of the new policy is the establishment of a tenure track for promising scientists which: a) establishes a clear-cut time line for the acquisition of tenure; b) requires that tenure track scientists be granted a recognized position as "NIH Investigator" with defined resources and research independence and c) provides for review of tenure track scientists at appropriate intervals. The new policy will allow the Director of the DIR to allocate tenure track positions more rationally and to track the progress of persons in those positions.

Tracking System for Animal Cost: In response to a need to carefully control costs of research involving animals, the DIR developed an extensive program to document the monthly and yearly costs of animals for each laboratory in the DIR. The new program will hold costs at the level of usage currently in effect and require individual Laboratory Chiefs to request and justify proposed increases in usage. In addition, it will allow re-allocation of under-used animal resources. This change is proving timely in view of increased animal studies involving transgenic and "gene-targeted mice.

Clinical Protocol Review Process: The DIR, in collaboration with Clinical Center staff, has established a computer-based system to calculate the costs of individual clinical projects. Henceforth, individual clinical projects will be reviewed at appropriate intervals by senior clinical scientists within the DIR to ascertain if a project is cost-effective. This process will allow the DIR to identify funds for new initiatives if the latter promise to provide a better use of increasingly scarce clinical resources.

Scientific Administration

Scientific Integrity Seminars: This year the DIR instituted a series of Scientific Integrity Seminars. These consisted of initial training seminars for Laboratory Chiefs and other senior DIR scientists conducted under the auspices of representatives of the NIH Office of Scientific Integrity. Subsequently, "grass-roots" seminars involving the entire scientific staff of DIR Laboratories were conducted under the leadership of senior scientists that had received initial training. Relevant subjects such as scientific fraud, authorship, data recording and responsibilities of scientific mentors were discussed. These Seminars will be held periodically over the coming year.

New Initiatives Committee: To aid in long-term planning within the DIR, a New Initiatives Committee was created to provide advice to the Director, DIR. This Committee, chaired by the Deputy Director, DIR, consisted of senior NIAID scientists (Section Heads, or Laboratory Chiefs on the Bethesda campus and at RML). It met at monthly intervals to discuss common research facility needs within DIR. Particular attention was given to the need to set up a Protein Purification and Expression Unit which will serve both the newly created Structural Biology Unit and the DIR as a whole. In addition, approaches to the creation/expansion of facilities to provide DNA sequencing were considered. Reports from the New Initiatives Committee were presented at the Laboratory Chiefs' Retreat in late July, 1993 and its recommendations will be acted upon in the current fiscal year.

Laboratory Chiefs' Retreat: The DIR conducted its third annual Laboratory Chiefs' Retreat at the Rockwood Manor Park Center in Potomac, MD. The theme of this year's retreat was "New Initiatives" and spirited and open discussion was conducted on a variety of new programs/facilities in planning or proposed for the DIR. Included was a discussion of the Gene Therapy Program, Gene Targeting Facility and DNA Sequencing Facility. In addition, discussion of a possible repository for gene-targeted and transgenic mice, as well as special animal needs of intramural scientists was held.

EEO Affirmative Action Activities

The DIR has continued to emphasize Equal Employment and Affirmative Action Programs. As part of this effort, it has conducted on-going discussions with Minority Senior Scientists Advisory Committee (MINSAC), a NIAID-wide group of minority scientists and administrators, who are seeking to develop new ways to increase minority and women representation in the DIR program. In a related vein, the Director and Deputy Director, DIR has interacted extensively with the newly appointed Advisor to the DIR for Women's Affairs, Dr. B.J. Fowlkes. Dr. Fowlkes has attended Laboratory Chiefs' meetings and retreats, and has conducted surveys of women's salaries and resources within DIR. As a result of these surveys, steps have been taken to equalize DIR women scientists salaries.

DIR again conducted its annual Introduction to Biomedical Research Program for minority students across the country. The program, jointly sponsored by the Office of the Director, NIAID, and the DIR was successful in bringing 54 students to NIH for personnel interaction with NIAID scientists for a two day lecture program. Thirteen students from this group as well as others from prior year programs, were selected for summer research positions in NIAID Laboratories and were thus the recipients of intensive one-on-one scientific training. In addition, seven other students obtained summer positions in other NIH institutes.

The DIR also continued to participate in the NIAID Adopt-a- School program with Dunbar Senior High School in Washington, DC. As part of this program, approximately eight DIR scientists went to Dunbar and provided 1-2 hours science seminars for Dunbar students. Selected Dunbar students then participated in the lecture program of the Introduction to Biomedical Research Program (as mentioned above) and 2 students were placed in summer positions in NIAID laboratories. Between the Introduction to Biomedical Research Program and the Adopt-a-School Program some 31 minority students were included in our summer research program, or 31/58 of the total summer program. Finally, the DIR expanded its productive collaboration with Howard University by which the two Institutions exchange patient care expertise. Within the context of this collaboration DIR physicians attended patient care rounds in general medicine and infectious diseases at Howard University in Washington, DC. Howard University, in turn, sent a fellow to NIH for a month-long inpatient rotation. Currently, DIR is setting up a joint program with Howard Universities physicians for the study of drug-resistant M. tuberculosis infections. This program will allow physicians and scientists at both institutions to conduct epidemiological studies and to provide a source of patient materials for on-going NIAID laboratory studies of M. tuberculosis.

Personnel Changes

This year two outstanding members of our staff, one scientific, one administrator left NIAID. Dr. Michael Kaliner, Head, Allergic Diseases Section, Laboratory of Clinical Investigation, left NIAID to take a position with the Washington Hospital Center as head of a newly formed Allergic Diseases Institute. Ms. MaryAnn Guerra, Chief, Administrative Management Branch, DIR and the Chief, Office of Technology Transfer, OD, NIAID left to take a position as Executive Officer, NHLBI. Ms. Karen Faunce, formerly of the Administrative Management Branch, NEI, was appointed the fill Ms. Guerra's position.

Board of Scientific Counselors

The NIAID Board of Scientific Counselors under the leadership of Dr. Dennis Kasper, reviewed the Laboratory of Parasitic Disease, the Laboratory of Malaria Research located at the Bethesda campus, and the Laboratory of Persistent Viral Diseases, the Laboratory of Microbial Structure and Function, the Laboratory of Vectors and Pathogens, and the Laboratory of Intracellular Parasites at our Rocky Mountain Facility. The reviews were generally excellent. NIAID thanks Dr. Dennis Kasper who completed his five year term on the Board, including a 2 year term as chair.

9/20/93

John I. Gallin, M.D. Date
Director, Division of Intramural
Research, NIAID

Office of the Clinical Director

National Institute of Allergy and Infectious Diseases

Annual Report 1993

Operations--Clinical Services

In FY 93, inpatient bed use increased steadily through the first 3 quarters and by years' end will total approximately 7,000 patient days. Outpatient visit counts to the 3 NIAID outpatient units on the 8th floor, 11th floor and Day Hospital similarly showed steady increases throughout the year and are predicted to exceed 10,000 visits. Of note, a concerted effort by several labs to shift patients from more costly inpatient stays to outpatient visits was partially responsible for the increased outpatient visit counts.

Inpatient Facilities

11 East closed for cosmetic and JCAHO_mandated fire and electrical renovations on April, 1993. It will reopen early in FY 94 with NIAID's Day Hospital, on-call room, and clinical personnel housed in 1/3 of the unit, and a Clinical Center-run general pediatrics unit in the remaining 2/3 of the unit. During the renovation, NIAID was the beneficiary of beds on 10E, 9W, 8W and 10W, borrowed from NEI, NICHD and NIAMS as well as the 18-bed complement on 11W and ran at occupancy rates averaging 70-80%.

11 West is scheduled for a similar code upgrade in FY 94. After its renovation, it will house 24 adult patients. Two rooms will be specially constructed for patients on respiratory isolation.

Outpatient Facilities

The NIAID Day Hospital, occupying 2 patient rooms on 11West, became an increasingly active part of the outpatient service with the 3rd and 4th quarter visit counts at over 200 per month.

The 11th floor clinic was renovated during the spring and summer, adding a procedure room and infusion room that NIAID will share with other institutes, as well as a new ENT suite. An office was constructed for Dr. John Bennett, Chief, Clinical Mycology Section, LCI and other rooms were assigned as office/exam rooms to LPD, LIR, and LCI. In recognition of our responsibilities to train fellows in Infectious Diseases and Allergy/Immunology and our desire to promote use of outpatient areas, additional clinic time was organized to be devoted to vasculitis and other clinical immunology patients.

Computer use in patient care and clinical research continues to be a major focus of the program. Desktop access to the CC Medical Information System (MIS), was made broadly available to many clinicians with MacIntosh computers via a connection with the NIAID LAN. The Clinical Center has replaced all older VMT MIS terminals with MacIntoshes on the inpatient units and has provided MacIntosh computers for the 11th floor clinic control desk and for the Fellows' Office on 11 West. A prototype clinical research database, called the Clinical Information Management Project (CIMP), continues development through a contract with Systex Corporation.

Clinical Protocol Review and Approval

The Multiple Project Assurance negotiated in 1993 granted final clinical protocol approval to the Deputy Director, Office of Intramural Research, NIH, through individual institute committees called Protocol Implementation Review Committee (PIRC). NIAID's PIRC is composed of the Scientific Director, Clinical Director, and the Director, Division of Allergy, Immunology and Transplantation and is responsible to review all protocols for scientific merit, public responsibility and NIAID resource use. Consequently, each protocol is subjected to a resource review by the Office of the Clinical Director concurrent with its NIAID Institutional Review Board (IRB) review, which is newly focused more directly on ethics and human costs of clinical research.

Infectious Diseases and Allergy/Immunology Training Program

The clinical training programs of NIAID in Infectious Diseases and Allergy/Immunology continue to attract well-prepared applicants. Dr. Michael Kaliner resigned after a long tenure as Director of the Allergy/Immunology Training Program, accepting a position to establish a practice and training program in allergic diseases at the Washington Hospital Center. Drs. Michael Polis and Judy Falloon assumed responsibilities as Co-Directors of the Infectious Diseases Training Program which remains under the overall direction of Dr. John Bennett.

Collaboration with Howard University

In late FY 91 and 92, a formal collaboration was developed between NIAID and Howard University, Washington DC, with the goal of mutually informative exchange of patient care expertise between the two groups. Senior NIAID physicians have attended and conducted patient care rounds on general medicine, infectious diseases and immunology patients. An attending rotation at Howard University was offered to NIAID staff members in FY 93. Howard University has sent one fellow for a month-long rotation on NIAID's inpatient service and is making plans for sending others in the coming medical academic year. Plans are underway to establish an NIAID/Howard University collaboration on the epidemiology and treatment of tuberculosis, including multiple drug resistant TB, in the inner city population in FY 94.

Clinical Accomplishments during FY 93

Interferon-gamma is being studied for its potential to enhance host defense in patients with X-linked susceptibility to Mycobacterium avium intracellular and for patients with Hyper-IgE-recurrent infection syndrome. A phase IV study of interferon-gamma in patients with chronic granulomatous disease continues.

FIAU, while demonstrating efficacy in suppressing chronic hepatitis B infection in HIV-infected patients and immunocompetent patients in short term dosing, resulted in severe hepatic toxicity among a group of patients receiving longer term dosing, causing the drug to be withdrawn from further testing. A randomized, controlled study of a candidate HSV2 vaccine was scheduled to begin in late FY 93. New anti-retroviral drugs being studied included U-90, a non-nucleoside analog reverse transcriptase inhibitor and PMEA, a nucleotide analog. A trail of Atovaquone (BW566C80), as treatment of cryptosporidiosis and microsporidiosis in HIV-infected patients was not effective. Foscarnet was shown to confer a survival benefit in a group of AIDS patients who received it as therapy for cytomegalovirus retinitis. A variety of immune-based therapies for HIV infection remain under investigation. Among these interferon-alpha, IL-2, and adoptive transfer of activated lymphocytes. Studies of treatment of Wegener's granulomatosis with low-dose methotrexate demonstrated that methotrexate may be an alternative therapy for patients with non-life-threatening illness or patients who experienced significant toxicity with cyclophosphamide therapy. Patients with filarial diseases and leishmaniasis were referred for evaluation, treatment and to provide research material for studies on the pathogenesis of parasitic diseases.

Observational studies conducted within the program continue to add to the body of knowledge of a variety of disorders. Chronic fatigue syndrome is being studied from neuroendocrinologic, immunologic and neuropsychiatric angles. A study of a clinical syndrome involving CD4 lymphopenia and opportunistic infections in HIV seronegative individuals showed the syndrome to be multifactorial in origin, without a retroviral etiology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00020-18

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies with PolyICLC

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Hilton B. Levy, Ph.D., Special Assistant to the Scientific Director, NIAID

COOPERATING UNITS *(if any)*

Dr. Andres Salazar, Walter Reed; Dr. Meir Kende, USAMRIID; Dr. J. Morales, Presbyterian Hospital, Puerto Rico; Dr. R. Meheshwari, USUHS; Dr. G. Dutta, Central Drug Research Institute, Lucknow, India.

LAB/BRANCH

Office of the Scientific Director (OSD)

SECTION

INSTITUTE AND LOCATION

NIH/NIAID, Building 14B South, Room 300, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0		0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are continuing to use PolyICLC in the treatment of human glioma. We now have 24 patients, many of which have been on for over 26 months. The grade 3 glioma (astrocytoma) are all well at 26 months with Karnofsky's score about 90. The median life expectancy of astrocytomas has been 18 months. Their tumors, as indicated by gadolinium enhanced M.R.I. are mostly shrinking, the grade 4 gliomas are not doing that well, although it appears that their life expectancy may be enhanced. Additional experiments with monkey malaria (P. cynomolgi) have been done. Treatment of monkeys with PolyICLC prevents malaria relapse, an important aspect of the pathology of human malaria. The study of the effect of PolyICLC on chronic progressive multiple sclerosis continues. Thirty-one patients have been on study, some for as long as 10 years. Some continue to decline, some stabilize and a few improve slightly. The EDSS score (measure of degree is disability) would be expected to decline about 1 point per year in this group of patients. The mean decline on all our patients has been 0.19 points/year.

The supply of PolyICLC is getting precariously short.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00594-02 ODIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Structure and Function of Fcγ and Fcε receptors

PRINCIPAL INVESTIGATOR

Jean Pierre Kinet, Section Chief - MAIS, OSD, NIAID
Anna Teresa Brini, Visiting Assoc.
Marie-Helene Jouvin, Visiting Assoc.
Helmut Kuster, Visiting Assoc.
Li Zhang, Visiting Assoc.
Odile Mejan, Visiting Fellow
Rosella Paolini, Visiting Fellow
Martin Adamczewski, Visiting Fellow
Kenichi Ochiai, Visiting Fellow
Gai Mi Lee, Laboratory Worker
Jami Willette-Brown, Microbiologist
Fumiyoski Takizawa, Visiting Fellow
Bernie Effertz, IRTA

COOPERATING UNITS
-Laboratory of Clinical Investigation, NIAID (Rottem, Metcalfe)
-Laboratory of Experimental Immunology, NCI (O'Shea, Kennedy, Ortaldo)
-Cell Biology and Metabolism Branch, NICHD (Orloff, Klusner)-John Hopkins University

LAB/BRANCH

Office of Scientific Director (OSD)

SECTION

Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION

NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
10.5	10	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK

This project was terminated. (October 1, 1990 to September 30, 1991)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00662-02 ODIR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Characterization of the FcεRI α chain by crystallography

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

Jami W. Brown	Microbiologist	MAIS, NIAID
Salvatore Sechi	Visiting Associate	MAIS, NIAID

COOPERATING UNITS

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

The receptor with high affinity for IgE (FcεRI) is a tetrameric complex of an IgE binding α chain, a β chain and a dimer of γ chains. In the past we have found that the extracellular region of the α chain is solely responsible for ligand binding. In order to further characterize the receptor binding site by crystallography, we have generated various transfectants capable of secreting large amounts of truncated α chain capable of binding IgE. However this material, like the native α is heavily glycosylated. This is preventing the formation of analyzable crystals. In order to circumvent this problem, we are following two different strategies: (1) The transfection of truncated α chain in cells with a defective glycosylation machinery. (2) The mutation of the seven N- linked glycosylation sites. Finally we are also attempting to produce a similar molecule in E. coli with the hope of using the NMR techniques to charaterize the receptor binding site.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00663-01 ODIR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Low affinity IgE receptors as receptors for IgE

PRINCIPAL INVESTIGATOR

PI:	Jean-Pierre Kinet, Section Chief	MAIS, NIAID	
Others:			
	F. Takizawa	Visiting Fellow	MAIS, NIAID
	M. Adamczewski	Visiting Fellow	MAIS, NIAID

COOPERATING UNITS

NONE

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

This project was terminated.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00664-02 ODIR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Distribution and role of FcεRI in human cells

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

K. Ochiai	Visiting Fellow	MAIS, NIAID
A. Brini	Visiting Associate	MAIS, NIAID
D. Dombrowicz	Visiting Fellow	MAIS, NIAID
V. Flamand	Special Volunteer	MAIS, NIAID

COOPERATING UNITS

-G. Stingl (Vienne, Austria)
-M. Capron (Lille, France)

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

We have generated a monoclonal directed against the α chain of the human FcεRI. With this reagent we found that FcεRI is expressed on the surface of Langerhans cells in human skin and we are attempting to determine the possible role of this receptor in atopic dermatitis. Furthermore, we found that FcεRI is expressed on the surface of monocytes of allergic individuals although its role on these cells is not yet understood. Finally, we detected FcεRI on eosinophils of patients with hypereosinophilia and found that this receptor is involved in eosinophil - mediated cytotoxicity against parasites. Our plan is now to cross transgenic mice expressing human α under the control of the human promoter, with the α gene knock-out mice which we have developed. This cross should generate a mouse expressing exclusively the human receptor and will help us in the analysis of the tissue distribution of FcεRI.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00665-02 ODIR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
The role of the FcεRI receptor subunits and of the receptor associated molecules in signal transduction

PRINCIPAL INVESTIGATOR
PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID
Others:

M. Adamczewski	Visiting Fellow	MAIS, NIAID
M. H. Jouvin	Visiting Associate	MAIS, NIAID
R. Paolini	Visiting Associate	MAIS, NIAID
B. Cuenod	Visiting Fellow	MAIS, NIAID
A. Valle	Visiting Fellow	MAIS, NIAID
R. Numerof	IRTA	MAIS, NIAID
A. Scharenberg	Special Volunteer	MAIS, NIAID

COOPERATING UNITS
NONE

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.0	7.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

The high affinity IgE receptor belongs to a class of receptor which lacks intrinsic enzymatic activity but activates non-receptor tyrosine and serine/threonine and phosphatases. This activations leads to phosphorylation and activation of phophatidylinositol-specific phospholipase C, an increase in intracellular calcium and ultimately the cell degranulation and the release of the mediators of allergic reactions.

We are attempting to identify the role of each individual receptor subunit in signalling and to define the kinases and phosphatases and the other signalling molecules interacting directly with the receptor after activation. We found that the src family tyrosine, lyn, associates with a tyrosine-containing amino acid motif of one of the cytoplasmic portion of the β subunit. Upon aggregation of the receptor, lyn becomes activated and phosphorylates the receptor β and γ subunits. Then, another kinase, syk, is activated by the γ subunit. Both kinases are required for full cell activation. We also found that the activity of these kinases is modulated by the phosphatase CD45. Finally, we discovered, that the receptor become ubiquitinated after activation and that this process is very rapid and reversible, suggesting a new signaling mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00666-01 ODIR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
The role of the molecules interacting with FcεRI in signal transduction

PRINCIPAL INVESTIGATOR
PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID
Others:

M. Adamczewski	Visiting Fellow	MAIS, NIAID	A. Brini	Visiting Associate	
B. Cuenod	Special Volunteer	MAIS, NIAID	R. Numerof	IRTA	MAIS, NIAID
L. Chung	IRTA	MAIS, NIAID			
R. Paolini	Visiting Associate	MAIS, NIAID			
A. Valle	Special Volunteer	MAIS, NIAID			

COOPERATING UNITS

NONE

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.9	4.9	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

This project was terminated and combined with project number: ZO1 AI 00665-02 ODIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00667-01 ODIR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Regulation of the expression of the human genes encoding FcεRI α and γ chains

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

A. Brini	Visiting Associate	MAIS, NIAID
F. Takizawa	Visiting Fellow	MAIS, NIAID
G. M. Lee	Laboratory Worker	MAIS, NIAID

COOPERATING UNITS

NONE

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS: 2.2	PROFESSIONAL: 2.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

This project was terminated.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-939

ANIMAL CARE BRANCH

ANNUAL REPORT FY 1993

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ANIMAL CARE BRANCH

1993 Annual Report

Summary

Much of the current research in the understanding and treatment of allergies and infectious diseases requires the use of laboratory animals. The care and treatment of these animals can have a profound affect on experimental results. An animal's environment, it's daily care, the presence or absence of disease-causing microorganisms, the amount of pain or distress that an animal experiences may affect the validity of research data generated through the use of animals. The NIAID's intramural scientists today are very sensitive to these factors and their ethical responsibility for ensuring that animals used in research receive high quality care. All NIAID scientists conducting research that involves animals are required to attend an NIH training course ("Using Animals in Intramural Research") that offers not only a broad perspective on current animal issues, but also provides specific information on actual techniques and procedures.

It is the responsibility of the NIAID's Animal Care Section and Animal Care Branch in Hamilton, Montana and the Metropolitan, D.C. area respectively to provide daily care to all animals maintained within NIAID animal facilities, to oversee NIAID's intramural contracts involving animals, and to assist NIAID scientists with animal-related problems. The NIAID maintains four animal facilities at the NIH campus in Bethesda, Maryland; one at the NIAID's Twinbrook II facility in Rockville, Maryland; one animal facility at the Frederick Cancer Research and Development Center (FCRDC) in Frederick, Maryland; and four animal facilities at the Rocky Mountain Laboratories (RML) in Hamilton, Montana. Approximately 59,000 square feet of space (26,000 sq. ft. at the NIH in Bethesda, Maryland; 3,500 sq. ft. at the FCRDC in Frederick, Maryland; and 29,000 sq. ft. in Hamilton, Montana), excluding pasture land, are devoted to the NIAID Animal Care Program.

The NIAID's Animal Care Branch and Animal Care Section provide guidance to the Institute's intramural scientists using animals in research projects. This guidance includes assistance in the purchase of animals, the selection and proper administration of anesthetics and analgesics, the diagnosis, treatment and control of infectious agents, and the performance of technical procedures in laboratory animals. The Animal Care Branch and Animal Care Section maintains production colonies of over ninety different strains of mice, hamsters, and rabbits for NIAID investigators within the Division of Intramural Research or contract animal facilities. Many of these animals are unavailable anywhere else in the world, or are available only after long delays. The NIAID's Division of Intramural Research is committed to the use of animals only when alternative methods are unavailable, and fully supports existing Federal rules and regulations pertaining to the care and use of animals in biomedical research.

ADMINISTRATIVE MANAGEMENT BRANCH

ANNUAL REPORT FY 1993

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ADMINISTRATIVE MANAGEMENT BRANCH, DIR
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1992 TO SEPTEMBER 30, 1993

INTRODUCTION

The Administrative Management Branch (AMB) is charged with the responsibility of planning, directing and coordinating administrative management functions for the Division of Intramural Research (DIR), NIAID. These functions include personnel, space, facilities and financial management, procurement and travel regulations.

PERSONNEL

During FY93 the AMB underwent extensive changes in administrative personnel. A new Chief of Financial Management and Administrative Services Section, AMB was appointed and, in turn, an Administrative Officer was hired who will serve as a focal point on all aspects of contract administration. In addition, new Senior Administrative Officers were appointed to Buildings 4, 10, and the Twinbrook II facility.

Additionally, a Stride Intern was selected to train in the Operations Support Services Section, AMB, and is currently stationed at the Twinbrook II administrative office. This is not only an excellent career development opportunity for an intern, but provides additional support to the DIR laboratory staff.

To better serve the needs of the DIR laboratories, a new Editor position was established in the AMB to assist the labs and administrative staff with their editorial requirements, such as manuscript preparation, annual reports, Board of Scientific Counselors Reviews, etc. This is a vital position in that it provides much-needed editorial skill in developing this documentation for publication and/or presentation at scientific meetings.

During FY93 the AMB participated in the Career Development and Exchange Program which allows for exchange of administrative staff members to other areas within the NIAID. AMB placed one of its purchasing agents on detail to the Contracts Management Branch, NIAID, and this exchange was such a success that this Branch hired the individual as a permanent employee!

A teambuilding and quality management process for the Rocky Mountain Laboratories AMB staff has been initiated, and it is anticipated that this TQM process will continue to enhance the customer service provided to the RML scientific staff. A similar training program is being implemented for all of AMB in the future.

BUDGET AND FINANCIAL MANAGEMENT

The FY93 DIR budget is approximately $123 million which is a 2.3 percent increase over the FY92 allocation. Forty percent of this amount was allocated to support AIDS and AIDS-related research. The AMB is charged with the responsibility for monitoring these funds in each specific research category.

In addition, the DIR received monies for special categories such as overseas initiatives, royalty income, CRADAs and Tuberculosis research. With the disbanding of the FAES, the Financial Management and Administrative Services Section and program administrative staff have been heavily involved in establishing new Gift Fund accounts within the NIAID accounting structure to encompass accounts previously within the FAES. These gift funds will allow flexibility in the purchase of supplies, services and equipment, as well as salary support.

The account reporting function at the Rocky Mountain Laboratories was streamlined to insure that account reporting to DFM in Bethesda and U.S. Treasury in San Francisco is done in a more timely manner. Travel management is now being handled on-site at RML with new, more efficient tracking systems established.

RENOVATIONS AND SPACE RELOCATIONS

The AMB was involved in several major renovations and relocations during FY93.

Building 10

The DIR has defined space in the 11E Patient Care Unit (PCU) to be used by the Clinical Center for a Pediatric Unit (4,526 square feet). Additionally, the Clinical Center has agreed to fund the renovations of the new NIAID Day Hospital (1,617 square feet) also located in the 11E PCU. This renovation is ongoing and is scheduled to be completed in October, 1993. Also, part of this renovation is a new office for the NIAID Clinical Director for which the NIAID is providing the funding. The DIR, NIAID, along with the Clinical Center and NCI have completed a renovation of the 11C Clinic. This renovation expanded the use of the 11th floor clinic allowing several NIAID physicians and nurses to have offices in the renovated space. The renovation of 11C103 has been completed and is now occupied by members of the Clinical & Molecular Retrovirology Section, Laboratory of Immunoregulation. The Laboratory of Clinical Investigation has renovated office space for a new "Cytokine Biology Section". The renovation is now complete and the space is occupied. The Laboratory of Clinical Investigation, Clinical Mycology Section has planned renovation of office space which is scheduled to be complete in August, 1993. The Laboratory of Clinical Investigation, Mucosal Immunity Section has nearly completed a laboratory/office renovation which is scheduled for completion in July, 1993.

Building 7

The Building 7 Animal Holding Facility passed it AAALAC accreditation in FY93. The Animal Care Branch (ACB) continues to upgrade the facility with changes to its physical environment, security, and the computer hardware and software support aspects of the facility. A new entrance system installed in Building 7 provides greater security to our animal rooms and was implemented as a recommendation from the last AAALAC inspection.

The 6B multi-user facility continues to be a integral part of the animal research studies being conducted by NIAID investigators and is currently being utilized near capacity.

The Hepatitis Viruses Section, Laboratory of Infectious Diseases completed a biological containment laboratory renovation in April 1993 to further advance and study the identification and cloning of both Hepatitis Virus C and Hepatitis Virus E by using Polymerase Chain Reactions (PCR) techniques which require a P2/P3 laboratory facility. This renovation was a high priority and accomplished in record time.

During the Summer of 1993, we will be completing renovations for the Virology and Cellular Immunology Section, Laboratory of Immunopathology. With incoming staff, the added improvements will greatly increase much-needed bench space to conduct the lab's scientific studies.

Building 4

A joint renovation project is underway with the Laboratories of Parasitic Diseases and Malaria Research. Members of the Physiology and Biochemistry Section of the LPD will be relocated from Building 4 to the Twinbrook II facility. This will allow for an expansion of the Laboratory of Malaria Research.

Twinbrook II

The Phase 1A renovation to provide Library, Seminar, Office and a lunchroom facility is continuing. Occupancy is expected in September, 1993.

The Phase 2 First Floor Laboratories are currently under construction with anticipated beneficial occupancy in October, 1993. This phase of renovation will include a new Structural Biology Laboratory, relocation of Intramural scientists from the Biological Resources Branch, Frederick, as well as various central support space for the entire facility. Expansion of the Animal Care Branch will include a walk-in high vacuum sterilizer to meet AAALAC requirements.

RML

The replacement of the facility smoke stack was successfully negotiated onto a "fast-track" with the Division of Engineering Services, with completion of the project anticipated for October, 1993.

PROCUREMENT/CONTRACTS

In order to better serve the NIAID, a major reorganization is underway which will create a new and separate Procurement Branch for NIAID, the Administrative Management Operations Branch (AMOB). The new Branch will include all delegated procurement and Research & Development functions for DIR, OD, and Extramural. Recruitment is ongoing for a new

DelPro Section Chief, Negotiated Contracts Section Chief, and Branch Chief for the AMOB. Concurrently, the DIR Purchasing Agents were organizationally as well as physically relocated in June, 1993 from the third to the first floor of the Solar Building. The new space will incorporate all staff members of the newly formed Branch and provide enhanced career development opportunities for the purchasing agent staff.

The Administrative Office located at Twinbrook II provides funding documentation for contract award, renewals, option year election and modifications for procurement contract support. Five contracts were awarded in FY93 and six are pending award before the close of CY93.

Several Indefinite Delivery contracts have been negotiated and will be in place by October 1993 in order to facilitate and expedite the procurement of research supplies for the laboratories.

Overall, procurement audits conducted by NIAID's internal review team and NIH reviewers were favorable with NIAID continuing to be commended for its purchasing practices.

AUTOMATION

In FY93 design and implementation of a migration from a 3Com 3Plus LAN to a Microsoft LAN Manager network environment is continuing. This includes server and workstation hardware and configurations.

A Microsoft NT is being evaluated for a future Network Operating system to coincide with, and eventually replace, the LAN Manager as the core Network Operating System for the NIAID LAN.

Network access has been implemented for Wylbur, Helix, Medlars and a variety of Internet services via a loadable TCP/IP protocol stack. This has greatly enhanced both the speed of data transmission and the scope of data access. It also negates the need for costly data lines/modems to access these services.

A Gopher Server which supports scientific and clinical databases worldwide was evaluated, designed and installed. This service can be accessed from either a PC or MacIntosh computer.

Design and implementation of the Clinical Information Management Project is continuing. Partial increments have been released and are in production. Increments adding more functionality will be available in the coming year.

Implementation of the new AMBIS 2000 system is scheduled for October, 1993.

A new client/server access to NIH resources is being evaluated.

Video conferencing and network supported were established for the newly occupied space in Building 10A.

The DIR-wide (and eventually NIAID-wide) Space Reporting System is on-going and in the programming stage. This system will allow greater flexibility and access to space management data within the laboratories and administrative offices and eventually across all NIAID space, both occupied and on contract. This system will also assist in the immediate identification of data problems relative to the NIH Space Management System, and enable AMB to prepare for reports of the Annual Management Fund Assessment and Clinical Center yearly assessment system.

Development of a protocol tracking system is continuing which will provide enhanced monitoring of clinical trials and protocols. This system will provide information on the cost of each active clinical protocol in order to present a clear picture of expenditures related to these activities.

Building 7 is currently fully cabled for the NIH-Wide LAN and NIAID Network which is supported by DCRT for future electronic communication uses.

The NIAID Administrative Office will be upgrading its hardware and software capabilities this Spring in order to keep up with technology and to better serve the scientific community by utilizing the many new reporting programs that are currently available for use on the NIAID LAN Network and required for personnel, administrative, managerial and budgetary purposes.

Administrative data management support was upgraded at RML with the installation of Status of Funds (SOF) and Employee Database System (EDS). The RML Personnel office has served as the initial testing office for on-line personnel management on the NIH-wide "Impact" system.

Biological Resources Branch
1993 Annual Report
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Annual Report
Biological Resources Branch
National Institute of Allergy and Infectious Diseases
October, 1, 1992 to September 30, 1993

RESEARCH PROGRESS

The Biological Resources Branch (BRB) investigates the structure and function of the genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and the molecular mechanisms controlling T-cell development. Special emphasis is placed on membrane receptors and differentiation antigens, in particular the family of adhesion molecules known as integrins, present on T and B lymphocytes, on the structures of major histocompatibility genes and proteins, on the nature of the peptides bound by class I and class II molecules, on the molecular basis by which antigens are processed and presented to T cells, on the *cis* elements and *trans* factors that regulate T cell receptor and class I gene expression and on proteins of the complement system. In addition to this fundamental immunological research, the BRB provides protein sequencing, peptide synthesis, and flow cytometry expertise and services for the DIR.

STUDIES ON THE MAJOR HISTOCOMPATIBILITY COMPLEX

Major histocompatibility complex (MHC) encoded class I molecules are integrally involved in the presentation of viral antigens, in the form of processed peptides, to cytotoxic T lymphocytes (CTL). Studies on the molecular basis of viral antigen processing and the nature of the interaction of processed antigen (peptides) with class I molecules and T cell receptors are emphasized in this research.

β_2 Microglobulin Dissociation Rates Reflect Class I/Peptide Complex Stability. Stable, recombinant, water-soluble complexes of HLA-A2 and HLA-B27 were reconstituted from ^{125}I labeled β_2-microglobulin, (β2m), a synthetic peptide and HLA heavy chains expressed in inclusion bodies of the *Escherichia coli* cytoplasm. Using this system, we were able to show that the dissociation rate of β_2m is an excellent measure of how tightly a given peptide binds to class I MHC molecules, that the ability to bind peptide is tightly coupled to the binding of β_2m and vice versa, and that regions of the molecule distal from the binding site influence the stability of peptide binding.

HLA-A3 Endogenous Peptides Define Anchor Residues. A motif specific to peptides that bind to the human class I major histocompatibility complex molecule HLA-A3 was identified by sequence analysis of HPLC fractions containing endogenous peptides. Twenty-six different sequences were obtained, 19 of which are nonamers. The majority of these endogenous peptide sequences contained Leu at position (P)2, while most sequences contained Tyr or Lys at P9. In addition, Phe was shared by 16 sequences at P3. Synthetic peptides corresponding to endogenous peptide sequences were shown to bind to HLA-A3. Six nonapeptides from within the influenza A nucleoprotein, matrix, and polymerase proteins, selected for synthesis based upon their possession of P2 and P9 anchor residues, were shown to bind HLA-A3. In contrast, none of a set of eight peptides that bound to HLA-A2, or six that bound to HLA-B27, bound detectably to HLA-A3. These findings provide a rationale for the design and selection of peptides that can be recognized by HLA-A3 restricted T cells.

Sequence Motifs Important for Peptide Binding to HLA-A2. Previous studies have indicated that most HLA-A2 binding peptides are 9 amino acid residues (aa) long, with a Leu at position 2 (P2), and a Val or Leu at P9. We compared the binding properties of different peptides by measuring the rate of dissociation of β_2-microglobulin from peptide-specific HLA-A2 complexes. The simplest peptide that we identified that could form HLA-A2 complexes had the sequence GLFGGGGGV, indicating that three nonglycine aa are sufficient for binding to HLA-A2. To determine whether most nonapeptides that contained Leu at P2 and Val or Leu at P9 could bind to HLA-A2, we tested the binding of nonapeptides selected from published HIV and melanoma protein sequences, and found that six of seven tested form stable HLA-A2 complexes. We identified an optimal antigenic undecapeptide from the cytomegalovirus gB protein that could form stable HLA-A2 complexes that contained apparent anchor residues at P2 and P11, indicating that the spacing between anchor residues can be somewhat variable. We conclude that the P2 and P9 anchor residues are of prime importance for peptide binding to HLA-A2. However, other peptide side chains (especially at P3) contribute to the stability of the interaction.

Specificity of Peptide Binding to Class I Molecules. To understand better the specificity of peptide binding by MHC class I molecules, we have evaluated the capacity of a panel of unrelated peptides to compete for the presentation of viral peptides presented by HLA-A3, HLA-A2, HLA-B37 and HLA-B27. Out of 41 peptides tested, only five bound to more than one of the MHC molecules analyzed. Pairwise comparisons of the peptide binding specificities among these four different class I molecules revealed no common competitor peptides in four of the six possible comparisons. Thus, each class I molecule appears to have a functionally distinct peptide binding site, as reflected by the ability to bind largely non-overlapping sets of peptides.

Cross-recognition of CTL Epitopes. Our studies showed that viral polymerase (PB2) peptides were recognized by nucleoprotien (NP)-specific CTL generated by NP-Vaccinia virus priming and influenza A virus stimulation, or NP peptide stimulation *in vitro* of NP-vac or influenza A-primed CTL. These results demonstrate that when screening peptide libraries one cannot assume that positive peptides necessarily identify the viral protein to which the CTL response is directed.

Molecular Studies on γδ T Cell Receptors and Adhesion Molecules (Integrins)

Soluble γδ T Cell Receptors. We have engineered chimeric proteins, containing the extracellular domains of the mouse Vγ1.1-Cγ4 and Vδ6.2-Cδ (V, variable; C, constant) TCR chains fused to the hinge region CH2 (H, heavy) and CH3 domains of human IgG1 heavy chain, and expressed them by transient transfection in COS cells. We showed that TCR γ-IgH and TCR δ-IgH chimeric chains are produced intracellularly in significant amounts, that the two chains can assemble correctly to form disulfide-linked, glycosylated heterodimers, and that a selective mechanism allows secretion of correctly paired receptor chains into the medium. Analysis with a clonotype specific Ab suggested that the fusion protein likely has a structural conformation similar to that of the native TCR. The COS cell expression system should prove useful for the production of additional TCR-IgH fusion proteins, and the subsequent analysis of TCR-peptide-MHC interactions.

Regulation of T Cell Receptor δ Gene Expression. To gain insight into the developmentally regulated expression of the mouse TCR Vδ-gene segments, we have investigated the role of the 5' promoter region of the Vδ1-gene. In vivo genomic footprinting localized several protein-DNA interactions to the stretch of DNA shown to have transcriptional activity. A computer analysis revealed that segments of DNA participating in these protein-DNA interactions were identical to the previously described cyclic AMP response element (CRE), E box, and leukemia virus E26 cis-acting elements. Transient transfection assays performed with constructs containing mutations at each of the localized cis-acting elements revealed that the CRE, E box, and Ets elements work together in driving promoter activity and that the CRE and Ets elements are the most important for driving transcription. Gel mobility shift analyses showed that each of these cis-acting elements is capable of binding specific nuclear factors present in Vδ1-expressing cells. These data indicate that multiple transcription factors acting in concert are responsible for Vδ1 gene expression.

B_1-Integrin Expression in the Developing Thymus. The expression, stucture, and function of VLAs (β_1 integrins) on mouse thymocytes were analyzed, as a first step toward understanding thier role in T cell development. Two major forms of β_1 were detected, which differed in their extent of N-glycosylation and sialylation. The larger (135 kDa, nonreduced) more basic β_1 was the only form on nonmature (J11d$^+$) adult thymocytes, with 135 kDa and larger β_1-chains expressed on fetal thymocytes from day 14 of gestation through birth. The smaller (120 kDa, non reduced), more acidic β-chain was found exclusively on mature (J11d$^-$) thymocytes and peripheral lymphocytes. VLA α-chain expression was also analyzed. Virtually all thymocytes were VLA-α_4^+, -α_6^+. VLA-α_5 was detected on both J11d$^+$ and J11d$^-$ thymocytes by immunoprecipitation. VLA-α_1 and -α_2 were immunoprecipitated only from cells within the J11d$^-$ population. Only J11d$^-$ thymocytes (12 to 15%) bound to fibronectin (via VLA-4 and VLA-5) and binding to laminin (via VLA-6) was two to fourfold higher in J11d$^+$ compared to J11d$^-$ thymocytes (60 to 80% vs 20 to 30%). These data show that major changes in VLA glycosylation, expression, and extracellular matrix binding (ECM) occur as thymocytes mature, supporting the hypothesis that VLA-ECM interactions play a role in T cell ontogeny.

PROTEINS OF THE COMPLEMENT SYSTEM

Studies are in progress to characterize the biochemical and biological properties of a plasma sialoglycoprotein (sgp120) that we first identified by its capacity to interact with the activated fourth component (C4b) of the classical complement pathway (CCP). Results to date support the idea that one form of this protein, sgp120-A, regulates the activation of the CCP. To obtain unequivocal data to support sgp120 as a new plasma protein and to gain information on its primary structure and function, studies are planned to complete cloning of the gene responsible for its synthesis. Pigtryin (PT) , a postulated analog of human inter-alpha-trypsin inhibitor (IATI) was shown to have 83% identity to sgp120 over 97% of its reported sequence. This translated to a peptide match of 76% identity with 81% similarity. Thus, PT is most likely the pig analog of human sgp120 and not IATI. PT has been identified as an acute phase reactant in a pig model of cardiogenic shock. Using a newly developed sensitive ELISA for sgp120 we have identified 2-3 fold increases in the serum sgp120 levels of burn patients suggesting that sgp120 like other complement components is an acute phase reactant.

SYNTHESIS OF PEPTIDE ANTIGENS

During the past year, almost 700 peptides have been produced by the Branch largely for defining MHC class I restricted T cell epitopes and for defining the nature of the interaction between peptides and class I molecules. A large number of peptides have also been used for preparing anti-peptide sera and for studying class II restricted T cell epitopes. Peptides have been used to prepare antisera to HIV-tax, cytokine receptors, diphenyl oxidase of *Cryptococcus neoformans*, Selectin-1, neutrophil cytochrome b558, SH2 domains of regulatory proteins, T cell receptors, Duffy receptor, *P. falciparum* tubulin, CD16 (FCγRIII-2), neutrophil chemotactic receptor, Herpes simplex viral proteins, HLA-DR antigens, mouse XLA protein, *Leishmania donovani* nuclease, Vaccinia virus proteins, human zap-70 and porcine syk tyrosine kinases, RAG-2, and Il-4 cytoplasmic domains; to study the function of ras- related G proteins, integrins, HIV-TAR RNA binding protein, human neutrophil and NADPH oxidase factors p47-phox and p67-phox, malarial circumsporozoite protein, human CD22 cytoplasmic domain, NADPH oxidase src domains, Herpes simplex virus attachment sites, and HIV superantigen effects; and to study CTL recognition of peptides associated with toxoplasmosis, myelin basic protein, which is possibly involved in multiple sclerosis, Friend Virus, Epstein Barr Virus, influenza viruses, and cytomegalovirus.

PROTEIN ANALYSES

The BRB determined the amino acid sequences of about 640 samples. About 440 of these analyses were for unknown protein or peptides and the remainder were for quality control of synthetic peptides. Most of the unknown sequences determined (about 250) were of endogenous peptides eluted from mouse d and b haplotype class I molecules (LI/NIAID, D. Margulies) and from human HLA-A3, -A2, -B27, -B14, -B8 and -A1 molecules (BRB). From these analyses, amino acid residues in peptides requisite for peptide binding to particular class I molecules have been determined. This information has been used to search protein sequence databanks for potential antigenic T cell epitopes that are recognized by cytotoxic T lymphocytes which has important implications for vaccine development. Using such an approach several new antigenic epitopes recognized by cytotoxic T lymphocytes specific for influenza virus proteins have been identified. In addition, proteins from a large variety of sources have been sequenced by the facility. These include *Crytococcal* enzymes and capsular proteins (Williamson, LCI); a filarial protective antigen (Nutman, LPD); *Toxo. gondii* antigenically active peptides (Sher, LPD); MHC class I endogenous and antigenic peptides (Margulies, LI); Malaria protein vaccine candidates (Miller and Kaslow, LMR); HIV integrase and uncharacterized HIV proteins (Theodore, LMM) and tumor asscoiated antigens (Seon, Roswell Park Memorial Cancer Institute).

FLOW CYTOMETRY ANALYSES

The FCS has recently required an ACAS 470 laser-based microscopic cytometer. This instrument is capable of quantitative fluorescent imaging on attached cells and cell selection for isolation of rare events. It is capable of two parameter analysis. We have also upgraded the computer component of the facility to provide: increased data storage and online availability, increased ease of remote connectivity of PC's and Mac's to the FCS computers; and compatibility with Microsoft Windows applications. The usage of the FCS instrumentation has increased 27% over FY '92, with sorting time remaining approximately 50% of annual usage. Total usage time was 3900 hours.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00169-16 BRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John E. Coligan, Ph.D., Chief, BRB/NIAID

Others:

K. Parker	S. Staff Fellow	BRB/NIAID
M. DiBrino	IRTA	BRB/NIAID
Y. Shirakata	V. Assoc.	BRB/NIAID

COOPERATING UNITS *(if any)*

Molecular Immunology Section, NINDS (W. Biddison)

LAB/BRANCH

Biological Resources Branch

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.1	3.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have made a detailed analysis of peptide properties important for forming class I/peptide/β_2m complexes using HLA-A2 as a model system. Preferential binding was for nonameric peptides having Leu or Met a position (P) 2 and Val or Leu a P9. The simplest peptide that we identified that could form HLA-A2 complexes had the sequence GLFGGGGGV, indicating that three nonglycine aa are sufficient for binding to HLA-A2. To determine whether most nonapeptides that contained Leu at P2 or Val or Leu at P9 could bind to HLA-A2, we tested the binding of nonapeptides selected from published HIV and melanoma protein sequences, and found that six of seven tested form stable HLA-A2 complexes, thus verifying the usefulness of these motifs for preditcing antigenic peptides. We identified an optimal antigenic undecapeptide from the cytomegalovirus gB protein that could form stable HLA-A2 complexes that contained apparent anchor residues at P2 and P11 (sequence FIAGNSAYEYV), indicating that the spacing between anchor residues can be somewhat variable. Finally, we tested the importance of every amino acid residue in the influenza A matrix peptide 58-66 (sequence GILGFVFTLL) for binding to HLA-A2, by using Ala-substituted and Lys-substituted peptides. We found that multiple positions contributed to stable binding, including P2, P3, P5-P7, and P9. Furthermore, we found that 1) Endogenous peptides form complexes that are about as stable as those formed with typical antigenic peptides. 2) Peptide exchange reactions proceed readily for complexes that have rapid β_2m dissociation rates. 3) The peptide exchange reaction does not require concomitant β_2m dissociation. 4) Distal parts of the class I molecule, which are not directly involved in peptide binding or β_2m binding, have a major impact on the stability of class I molecules.

A motif specific to peptides that bind to the human class I major histocompatibility complex molecule HLA-A3 was identified by sequence analysis of HPLC fractions and showed that the majority contained Leu at position (P)2 and most contained Tyr or Lys at P9, and, Phe was common at P3. Six nonapeptides from within the influenza A nucleoprotein, matrix, and polymerase proteins, selected for synthesis based upon their possession of P2 and P9 anchor residues, were shown to bind HLA-A3 but not other class I molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00172-15 BRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis of Peptide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John E. Coligan, Ph.D., Branch Chief, BRB/NIAID

Carl Hammer, Ph.D., Senior Investigator, BRB/NIAID
Jan Lukszo, Ph.D., Visiting Scientist, BRB/NIAID

COOPERATING UNITS *(if any)*

Center for Disease Control (Dr. Renu Lal); NICHHD (Dr. K. Ozato); NIHBL (Dr. W. Leonard)

LAB BRANCH

Biological Resources Branch

SECTION

Protein Analysis and Synthesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	1.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past year, almost 700 peptides have been produced by the Branch largely for defining MHC class I restricted T cell epitopes and for defining the nature of the interaction between peptides and class I molecules. A large number of peptides have also been used for preparing anti-peptide sera and for studying class II restricted T cell epitopes. Peptides have been used to prepare antisera to HIV-tax, cytokine receptors, diphenyl oxidase of *Cryptococcus neoformans*, Section-1, neutrophil cytochrome b558, SH2 domains of regulatory proteins, T cell receptors, Duffy receptor, *P.falciparum* tubulin, CD16 (FCγRIII-2), neutrophil chemotactic receptor, Herpes simplex viral proteins, HLA-DR antigens, mouse XLA protein, *Leishmania donovani* nuclease, Vaccinia virus proteins and human zap-70 and porcine syk tyrosine kinases, RAG-2, and Il-4 cytoplasmic domains; to study the function of ras-related G proteins, integrins, HIV-TAR RNA binding protein, human neutrophil and NADPH oxidase factors p47-phox and p67-phox, malarial circumsporozoite protein, human CD22 cytoplasmic domain, NADPH oxidase src domains, Herpes simplex virus attachment sites, and HIV superantigen effects, and to study CTL recognition of peptides associated with toxoplasmosis, myelin basic protein, which is possibly involved in multiple sclerosis, Friend Virus, Epstein Barr Virus, influenza viruses, and cytomegalovirus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00352-11 BRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John E. Coligan, Ph.D., Branch Chief, BRB/NIAID
Others: K. Sturmhoefel, Visiting Fellow, BRB, NIAID
A. Chang, IRTA, BRB/NIAID
S. Wadsworth, S.S. Fellow, BRB/NIAID
M. Halvorson, IRTA, BRB/NIAID
S. Otto, IRTA, BRB/NIAID
F. Martinon, V. Fellow, BRB/NIAID
W. Magner, IRTA, BRB/NIAID
G. Kikuchi, S.S.Fellow BRB/NIAID

COOPERATING UNITS *(if any)*

Laboratory of Immunology, NIAID (Dr. Ethan Shevach)

LAB/BRANCH

Biological Resources Branch

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.9	4.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The expression, structure, and function of VLAs (β_1 integrins) on mouse thymocytes were analyzed, as a first step toward understanding their role in T cell development. Two major forms of β_1 were detected, which differed in their extent of N-glycosylation and sialylation. The larger (135 kDa, nonreduced) more basic β_1 was the only form on nonmature (J11d$^+$) adult thymocytes, being expressed on fetal thymocytes from day 14 of gestation through birth. The smaller (120 kDa, non reduced), more acidic β-chain was found exclusively on mature (J11d$^-$) thymocytes and peripheral lymphocytes. VLA α-chain expression was also analyzed. Virtually all thymocytes were VLA-α_4^+, -α_6^+. VLA-α_5 was detected on both J11d$^+$ and J11d$^-$ thymocytes by immunoprecipitation. VLA-α_1 and -α_2 were immunoprecipitated only from cells within the J11d$^-$ population. Only J11d$^-$ thymocytes (12 to 15%) bound to fibronectin (via VLA-4 and VLA-5) and binding to laminin (via VLA-6) was two to fourfold higher in J11d$^+$ compared to J11d$^-$ thymocytes (60 to 80% vs 20 to 30%). The high percentage of J11d$^+$ thymocytes adhering to laminin suggest that VLA-6 exists in an activated state on most thymocytes, in contrast to resting peripheral T cells. These data show that major changes in VLA glycosylation, expression, and extracellular matrix binding (ECM) occur as thymocytes mature, supporting the hypothesis that VLA-ECM interactions play a role in T cell ontogeny.

We have engineered soluble chimeric TCR/IgG, containing the extracellular domains of the mouse Vγ1.1-Cγ4 and Vδ6.2-Cδ (V, variable; C, constant) TCR chains fused to the hinge region CH2 (H, heavy) and CH3 domains of human IgG1 heavy chain, and expressed them by transient transfection in COS cells. To gain insight into the developmentally regulated expression of the mouse TCR Vδ-gene segments, we have investigated the role of 5' promoter region of the Vδ1-gene. In vivo genomic footprinting localized several protein-DNA interactions to the stretch of DNA shown to have transcriptional activity. A computer analysis revealed that segments of DNA participating in these protein-DNA interactions were identical to the previously described cyclic AMP response element (CRE), E box, and leukemia virus E26 cis-acting elements. Transient transfection assays revealed that the CRE, E box, and Ets elements work together in driving promoter activity and that the CRE and Ets elements are the most important for driving transcription. These data indicate that multiple transcription factors acting in concert are responsible for Vδ1 gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00522-06 BRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Sequence Analyses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John E. Coligan, Ph.D., Branch Chief, BRB/NIAID

COOPERATING UNITS *(if any)*

LIR/NIAID, (H. Ehrenreich), LCI/NIAID (P. Williamson), LI/NIAID (D. Margulies) Roswell Park Memorial Inst. (B. Seon), LIG/NIAID (E. Long), LPD/NIAID (A. Sher, T. Nutman), LMR/NIAID (L. Miller, D. Kaslow), LMM/NIAID (T. Theodore)

LAB/BRANCH

Biological Resources Branch

SECTION

Protein Analysis and Synthesis Section

INSTITUTE AND LOCATION

NIAID/NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	0.2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The BRB determined the amino acid sequences of about 640 samples. About 440 of these analyses were for unknown protein or peptides and the remainder were for quality control of synthetic peptides. Most of the unknown sequences determined (about 250) were of endogenous peptides eluted from mouse d and b haplotype class I molecules (LI/NIAID, D. Margulies) and from human HLA-A3, -A2, -B27, -B14, -B8 and -A1 molecules (BRB). From these analyses, amino acid residues in peptides requisite for peptide binding to particular class I molecules have been determined. This information has been used to search protein sequence databanks for potential antigenic T cell epitopes that are recognized by cytotoxic T lymphocytes which has important implications for vaccine development. Using such an approach several new antigenic epitopes recognized by cytotoxic T lymphocytes specific for influenza virus proteins have been identified. In addition, proteins from a large variety of sources have been sequenced by the facility. These include *Crytococcal* enzymes and capsular proteins (Williamson, LCI); a filarial protective antigen (Nutman, LPD); *Toxo. gondii* antigenically active peptides (Sher, LPD); MHC class I endogenous and antigenic peptides (Margulies, LI); Malaria protein vaccine candidates (Miller and Kaslow, LMR); HIV integrase and uncharacterized HIV proteins (Theodore, LMM) and tumor asscoiated antigens (Seon, Roswell Park Memorial Cancer Institute).

We examined the effect on thrombin on astrocytic endothelins and report that endothelin-1 is released into the culture fluid in response to thrombin treatment. Astrocytic production of endothelin-1 appeared to be postranscriptionally regulated. We also found that two endothelin receptor genes (ET_A and ET_B) were transcribed in primary astrocyte cultures and that ET_A mRNA was down regulated in response to thrombin and entothelin-1. These studies indicate a role for thrombin in the regulation of brain perfusion through astrocytic endothelin-1 expression. In an attempt to explore the potential role of endothelins in HIV infection, we investigated the effect of the HIV-1 glycoprotein 120 on monocytic endothelin-1 production. We found that glycoprotein 120, similar to LPS, stimulates the secretion of endothelin-1, as well as TNF-α, from macrophages in a concentration-dependent manner. Using reverse transcriptase polymerase chain reaction, we found that circulating monocytes in HIV-infected individuals show a distinct expression of the endothelin-1 gene that is not detectable in healthy controls, indicating chronic activation of this gene in HIV-infection. In addition, cerebral macrophages in patients with HIV encephalopathy were strongly positive for endothelin. Thus, monocytic endothelins appear to be stimulated during HIV infection. Their potent vasoactive properties render them potential candidates for mediating alterations in the cerebral perfusion pattern associated with the AIDS dementia complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00523-06 BRB

PERIOD COVERED

October 1, 1993 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Flow Cytometric Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kevin L. Holmes, Ph.D., Head, Flow Cytometry Section

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biological Resources Branch

SECTION

Flow Cytometry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.25	.25	5.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The FCS has recently acquired an ACAS 470 laser-based microscopic cytometer. This instrument is capable of quantitative fluorescent imaging on attached cells and cell selection for isolation of rare events. It is capable of two parameter analysis.

We have also upgraded the computer facility to provide: increased data storage and online availability; increased ease of remote connectivity of PC's and Mac's to the FCS computers; and compatibility with Microsoft Windows applications.

The usage of the FCS instrumentation has increased 27% over FY '92, with sorting time remaining approximately 50% of annual usage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00543-06 BRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Recognition of Peptide Antigens by Virus-specific Cytotoxic T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John E. Coligan, Branch Chief, BRB/NIAID

Others:

M. DiBrino	IRTA	BRB/NIAID
M. Shields	IRTA	BRB/NIAID

COOPERATING UNITS *(if any)*
Neuroimmunology Branch, NINCDS (W. Biddison), LVD/NIAID (J. Bennik, J. Yewdell)

LAB BRANCH
Biological Resources Branch

SECTION
Molecular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

To understand better the specificity of peptide binding by MHC class I molecules, we have evaluated the capacity of a panel of unrelated peptides to compete for the presentation of viral peptides presented by HLA-A2, HLA-A3, HLA-B27, and HLA-B37. Out of 41 peptides tested, only five bound to more than one of the MHC molecules analyzed. Pairwise comparisons of the peptide binding specificities among these four different class I molecules revealed no common competitor peptides in four of the six possible comparisons. Thus, each class I molecule appears to have a functionally distinct peptide binding site, as reflected by the ability to bind largely non-overlapping sets of peptides.

In understanding CTL anti-viral responses and in creating vaccines designed to elicit CTL responses, it is critical to identify the portions of viral proteins that bind class I molecules and that are recognized by T cell receptors. Previous findings have indicated that a significant portion of the CTL response of H-2^d mice to influenza virus is specific for one of the viral polymerases (PB2). To identify the region of PB2 naturally processed and presented by influenza virus-infected mouse cells to CTL, 31 PB2 peptides of 9-16 residues in length were chosen and chemically synthesized. Two peptides, PB2, residues 146-159 and 187-195, were found to sensitize histocompatible target cells for recognition by influenza virus-specific CTL. When CTL were generated to individual viral proteins using influenza-vaccina recombinant viruses, we found, to our surprise, that PB2-specific CTL failed to recognize cells sensitized with PB2 peptides 146-159 and 187-195. Further analysis showed that these PB2 peptides were, in fact, recognized by nucleoprotein (NP)-specific CTL generated by NP-vac virus priming and influenza A virus stimulation, or NP peptide stimulation *in vitro* of NP-vac or influenza A-primed CTL. These results demonstrate that when screening peptide libraries one cannot assume that positive peptides necessarily identify the viral protein to which the CTL response is directed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00595-03 BRB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Purification, Characterization and Function of Proteins of the Complement System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Carl Hammer, Ph.D. Senior Investigator, BRB/NIAID

Others:

M. Knierman	Chemist	BRB/NIAID
T. Leto	Sr. Staff F.	LHD/NIAID

COOPERATING UNITS (if any)

Michael M. Frank, Chairman, Dept. of Pediatrics, Duke Univ., Med. Ctr., Durham, NC 27710

LAB/BRANCH

Biological Resources Branch

SECTION

Protein Analysis and Synthesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies are in progress to characterize the biochemical and biological properties of a plasma sialoglycoprotein (sgp120) that we first identified by its capacity to interact with the activated fourth component (C4b) of the classical complement pathway (CCP). Results to date support the idea that one form of this protein, sgp120-A, regulates the activation of the CCP. Sgp120 is also cleaved by kallikrein of the intrinsic coagulation-kinin generating pathway to produce protein fragments that may possess biological activity. To obtain unequivocal data to support sgp120 as a new plasma protein and to gain information on its primary structure and function, studies are planned to complete cloning of the gene responsible for its synthesis. Excellent agreement was obtained by computer isoelectric point prediction analysis of the combined translated amino acid sequence of D and G clones with the splice site (pI = 6.36, 758 residues) and without (pI = 5.76, 723 residues) to that obtained by direct electrophoretic analysis of desialidated sgp120-A/I, respectively. This supports our hypothesis that the larger C4b binding form, sgp120-A, is a product of a spliced gene such as in clone D, the insert representing the C4b binding domain. Antibody was produced to a peptide deduced from the C-terminal portion of the splice site from clone D and will be used to study the binding of sgp120-A to surface bound C4b as well as to the free component. Pigtryin (PT), a postulated analog of human inter-alpha-trypsin inhibitor (IATI) was shown to have 83% identity to sgp120 over 97% of its entire partially reported sequence. This translated to a peptide match of 76% identity with 81% similarity. Thus, PT is most likely the pig analog of human sgp120 and not IATI. The mRNA of PT, identified as an acute phase reactant in a pig model of cardiogenic shock, is increased > 5 fold. Using a newly developed sensitive ELISA for sgp120 we have identified 2-3 fold increases in the serum sgp120 levels of burn patients suggesting that sgp120 like other complement components is an acute phase reactant.

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1993 Annual Report
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Summary Report
Laboratory of Clinical Investigation
October 1, 1992 to September 31, 1993

Stephen E. Straus M.D.
Chief, Laboratory of Clinical Investigation
NIAID

Introduction

The LCI has had a long and productive history as a multi-disciplinary department in which both clinical and basic research studies are conducted dealing with fundamental problems in allergy, immunology, and infectious diseases. It has been, and remains, a dominant intramural program as regards training of clinician investigators and a prominent user of the exceptional in-patient and out-patient facilities of the NIH clinical center.

Senior staff continue to rise in international prominence and recognition, with countless invited lectures, appointments, and prestigious awards. LCI senior staff direct both the allergy/immunology and infectious disease training programs. They essentially constitute the Allergy Consult Service and direct and heavily staff the Infectious Disease Consult Service. Ten (10) LCI staff members serve in rotation as attending physicians on the in-patient units.

Organizational Resources

FY 93 represented the second full year in which the LCI has been headed by Dr. Stephen Straus. These first two years witnessed a marked alteration in the resources and programmatic direction of the Laboratory. The philosophy underlying the changes in the LCI encompasses several items:

1. To reduce slightly the size of some of the dominant older research sections.

2. To encourage the departure of staff not worthy of tenure or outside of the programmatic direction of the Laboratory.

3. To recruit a cadre of new, promising young investigators.

4. To select new tenure-track investigators who can excel in subject areas that best compliment existing strengths within the Laboratory.

5. To move components of the Laboratory from a

   strongly biochemical focus to a more molecular and cellular one.

6. To commit specific members of the Laboratory to full time clinical investigation while freeing others to full-time bench efforts.

Over the past two years notable achievements have been made towards each of these goals. First, the **Medical Virology Section** took over the large laboratory space previously occupied by Dr. Michael Frank. Of the seven (7) research modules vacated by Dr. Frank, two are committed fully to Dr. Jeffrey Cohen, a tenure-track Investigator, and one and one half modules were designated for common tissue culture and equipment space for the Section.

The opportunity for the Section to engage in imaginative basic virologic research efforts was heightened by recruiting two experienced investigators as non tenure-track Senior Staff Fellows. Dr. Liyanage Perera DVM, Ph.D. and Dr. Richard Williams, Ph.D. They bring to the LCI a combined fourteen (14) years of post-doctoral research experience in virology. In addition, two research nurses were recruited for full-time support of clinical trials involving genital herpes vaccine and chronic fatigue syndrome.

The **Clinical Mycology Section** moved this past year to occupy a smaller research space (5 modules versus 6) with balanced resource commitment to Dr. Kwon-Chung. An additional office in the clinic was committed for Dr. John Bennett in support of his expanding clinical protocol developments. The capacity of that Section to move its efforts regarding the pathogenesis of fungal infections to a more molecular level was further strengthened through the recruitment this past year of a molecularly trained Staff Fellow Dr. Yun Chang.

The **Mucosal Immunity Section** has sustained a highly productive research effort. To enhance its strengths and to expand its potential to extend fundamental discoveries into more molecular areas, efforts are underway to recruit a new independent staff member to the Section to share the existing resources. A formal search committee has been established and applications were sought through international advertising. To date over 60 candidates were considered and several formal interviews were conducted. It is expected that a selection will be completed during the Summer of 1993.

Dr. Michael Kaliner, the head of the **Allergic Diseases Section**, head of the Asthma and Allergy Center and head of Allergy/Immunology Training Program has now completed twenty (20) years of extraordinarily productive service to NIAID and to the allergy community. He has elected to retire from the U.S. Public Health Service and to establish an innovative new allergy center in the Washington Metropolitan area. It is expected that he will maintain active interactions with the NIAID clinical training effort. His pending departure permits the redirection of the remaining allergy/immunology efforts within the LCI. To this end, two senior staff fellows, Dr. Robert Hohman and Dr. Martha White left NIAID this year along with their technicians and all but four of the Section's fellows.

The space vacated by Dr. Hohman has been committed to a new independent investigator, Dr. Robert Seder, who moved to the LCI in the Summer of 1993 to form the **Lymphokine Regulation Unit**. He has completed four (4) years of prolific efforts in the Laboratory of Immunology were he worked with Dr. William Paul to define the functional implications of IL-4 on the regulation and activity of T cells of Th1 and Th2 phenotypes.

Dr. Seder has trained in internal medicine and in the coming year he will fulfill training obligations for the conjoint subspecialty boards in Allergy and Immunology. This would permit Dr. Seder to independently develop and actualize research efforts aimed at better defining the cellular regulatory features of chronic allergic, immune, and infectious disorders.

This past year Dr. Joshua Farber was recruited to the LCI to establish the **Cytokine Biology Unit**. Until May 1993 Dr. Farber was an assistant Professor in the Division of Infectious Diseases at Johns Hopkins University School of Medicine. There, he accrued independent funding to support fundamental investigations of macrophage proteins that are synthesized and released in response to cellular activation with LPS and gamma-interferon. He has identified and characterized molecularly, biochemically, and biologically, a series of proteins that are expressed by activated mouse and human cells. Dr. Farber will teach and hopefully inspire young clinical investigators in his laboratory and through ward attending duties, but he will have no clinical research obligations.

The LCI has been a vigorous proponent of the new tenure-track policy of the NIH and has followed its philosophy in practice even before its formal adoption campus-wide. Fully independent resources have been committed to Dr. Russo, Dr. Cohen, Dr. Farber, and Dr. Seder. In FY 93 both Dr. Russo and Dr. Cohen underwent formal mid-tenure reviews aimed at ascertaining their progress and potential and it suggested alterations in their efforts as appropriate to their levels of performance. It is intended that formal mid-tenure reviews will be performed for Dr. Farber and Dr. Seder as well in about three (3) years. The Board of Scientific Counselors will review the LCI again in May 1994 at which time their guidance will be important for the further consideration of the LCI's new programs and tenure-track candidates.

The period of revision of intramural space commitments on the 11th floor of the Clinical Center is drawing to a close. This past year witnessed a reassignment of both basic and clinical research space to the LCI. This has also been a period of budgetary restraint with only a 1% net increase in total Laboratory expenditures, which in the face of a several-fold higher inflationary cost for scientific equipment and supplies, translates into a net loss in real dollars for research. This past year, and in all likelihood for the foreseeable future, greater efforts than ever will need to be placed on prioritizing research efforts and on closely scrutinizing those that are sustained. Scrutiny of expenditures has extended this past year also to clinical resource utilization. The LCI and other clinical laboratories have commonly diminished their use of in-patient beds and is increasing its efforts in out-patient and day hospital based protocols.

Research Progress

Despite a great deal of relocations and physical renovations and departures of staff the stream of intense research productivity and excellence has remained unbroken. The following are broad summaries of the achievements of each of the components of the LCI:

The **Lymphokine Regulation Unit** was formed as an independent laboratory under the direction of Dr. Robert Seder with the goal of conducting and orchestrating cellular immunologic studies to better define the pathogenesis and immune disregulation of chronic allergic, immune and infectious disorders.

The **Cytokine Biology Unit** was formed in mid 1993 to support and extend Dr. Joshua Farber's imaginative work on how macrophages respond to inflammatory and infectious stimuli with the goal of identifying new targets for therapeutic interventions. He has already relocated and initiated a key series of research collaborations and continues a steady pace of publication.

The overall effort in the Unit is to identify and understand macrophage proteins that are important for immune and inflammatory responses. Dr. Farber began his work at Johns Hopkins University by identifying from within a cDNA library 11 genes whose transcription is strongly increased following exposure of mass macrophages to gamma interferon. 2 of these 11 genes were previously unrecognized and serve as the groups continuing focus. 1 of the genes known as CRG-2 is activated in response to other cellular stimuli but a mouse gene known as MuMig and human counterpart HuMig respond specifically to gamma interferon. The gene is also upregulated in mice injected with gamma interferon. The proteins for each of these interferon-induced genes have been purified and analyzed. Antibodies have been raised to them as well. Current efforts are directed at identifying the MuMig/HuMig receptors.

Research projects in the **Medical Virology Section** cover a range of issues, primarily ones dealing with the molecular biology, pathogenesis and treatment of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections although efforts regarding Epstein-Barr virus (EBV) and hepatitis B virus (HBV) and chronic fatigue syndrome (CFS) are mounted.

Regarding VZV infections, most of the program is directed at defining the ryes of specific viral genes and their promoters. Under the direction of Dr. Jeffrey I. Cohen detailed analyses of VZV regulatory genes 4, 10, 61, and 62 are under way. Several of these studies are being carried out by Dr. Hiro Moriuchi. Prominent findings include the termination of the activation demands of genes 61 and 62 and evidence that the gene 10 product also possesses transactivating function in contrast to a prior report. Dr. Liyanage Perera independently arrived at similar findings with regard to the gene 62 activation demand and he is studying in detail the promoter elements of this critical gene.

A major achievement to the past year has been the successful development by Dr. Cohen of a versatile system for manipulating the VZV genome so as to insert or delete sequences of interest. Using a library of 4 infectious overlapping cosmids spanning the genome he has succeeded in inserting the gene for β-galactosidase in VZV, rendering easy color identification of infected cells, of deleting genes and coding the viral ribonucleotide reductase and thymidylate synthetase and inserting elements to interfere with the coding sequences of genes 10, 61, and others. In addition to defining genes which are essential for replication this system is being used to insert heterologies genes so that the varicella vaccine could induce protective immune responses against other agents as well. To further study the various mutations made in VZV a guinea pig model of varicella is under development by Dr. Cohen and Dr. Perera.

Dr. Jeffrey Meier completed and published an exhaustive series of studies extending area observations of VZV gene expression in latently infected human sensory nerve ganglia. He showed specific expression of genes 29 and 62 and non-neuronal cells. Dr. Rhonda Kost will be attempting to extend these findings by in-situ hybridization and in-situ PCR analysis of human tissues. Studies of latently infected guinea pig tissues will also be undertaken.

Dr. Kost has also been characterizing gene 63 transcription and is studying responsiveness of it's promoter to transactivators as well as the regulatory function of the gene product itself. Dr.

Meier has defined a novel regulatory mechanism involving the promoter for genes 28 and 29. Expression of these transcriptionally divergent genes in productive infection is controlled by binding of a USF-like nuclear factor to a palindromic element in the antigenic domain.

During the past year Dr. Richard Williams established a new project to study the interactions between VZV and cell surface proteins. To date, he showed that VZV envelope proteins react with cells through heparin-bearing intermediates. The goal of this project is to define the specific and non-specific binding events that permit virus infection.

Studies of HSV-2 latency are being conducted and several new directions by Dr. Kening Wang. She is performing detailed deletional and mutational analysis of the latency-associated transcript promoter domain as well as transient expression assays, electrophoretic mobility shift assays, DNase footprint and methylation protection assays. A series of 4 upstream elements have been defined and are under scrutiny.

In an EBV-related project, Dr. Cohen has shown the low of viral EBNA to EBNA-2 antigen to effect lymphogenesis in the SCID-hu mouse model.

Several important findings were made in the Section's clinical studies. Trials of a new anti-viral drug for hepatitis B virus are being conducted by Dr. Robin McKenzie in collaboration with scientist in NIDDK. 9 of 24 patients with chronic active HBV infection lost circulating viral DNA following well tolerated treatment with oral fluroiodoarauracil.

Dr. McKenzie is also directing a controlled trial of low doses of the steroid hydrocortisone for treatment of CFS. 33 of the intended 70 patients are on study. In related efforts, Ms. Dale in collaboration with Dr. Warren Strober and Dr. Scott Fritz are conducting detailed cellular immunonalysis of CFS patients. Prior observations of increased circulation of memory T cells are being verified with new patients and extended to in-vitro studies of purified cells and their production of cytokines in response to simulation.

A major achievement of the Section this past year was the completion and analysis of a placebo-controlled study of the recombinant glycoprotein D vaccine for frequently recurring genital herpes. In collaboration with scientists at the University of Washington 98 patients with frequently recurring genital herpes were enrolled and vaccinated with vaccine or placebo and all recurrences were documented. Upon study closure the data revealed a statistically significant reduction in frequency of recurrences in the glycoprotein vaccine recipients. These patients had an average of a 30% lower rate of documented recurrences than placebo recipients. This is the first study to verify the immunotherapeutic potential of a vaccine, a finding with broad implications for other chronic viral infections including HIV.

These studies are being extended and will be verified in the coming years. First, studies are under way using a new vaccine with a combination of glycoprotein D and glycoprotein B in a lipid adjuvant known as MF59. This vaccine is being incorporated into a larger placebo-controlled trial beginning in FY93 to verify and extend the results of the previous immunotherapeutic trial.

Dr. Dean Metcalfe and his colleagues in the **Mast Cell Physiology Section** are conducting a series of highly integrated laboratory and clinical investigations of mast cell biology and the pathogenesis of systemic mast cell disorders. The dominant effort involves studies of the biochemical and cellular requirements of mast cells for growth and differentiation. Members of the Section have the capacity now to grow human and rodent mast cells in culture and to derive mast cells from $CD34^+$ progenitors in the presence of IL-3. Stem cell factor (c-kit ligand) further potentiates the growth of mast cells. IL-3 also appears to prevent cultured mast cells from undergoing apoptosis, or programmed cell death, and the stem cell factor has the capacity to independently prevent death upon withdrawal of IL-3. Mutant mast cells with defective c-kit will die despite addition of it's ligand.

The requirements by mast cells for both IL-3 and c-kit ligand and the differential roles of each of these factors is an important issue for study. The c-kit ligand is released by connective tissue cells leading not only to growth of mast cells but also in the specific adhesion to tissue proteins. Several in the Section have now verified that mast cells adhere spontaneously to vitronectin and are induced by c-kit ligand to adhere to fibronectin. These mechanisms are likely to be responsible for the physiologic localization of mast cells in tissues. They also illuminate pathways that could be abnormal in systemic mastocytosis, in which there is pathologic proliferation of mast cells in tissue.

It is evident that mast cells participate in allergic and inflammatory disorders through the release of chemical mediators in response to specific cellular activation signals. A key project in the Section is examining molecular events following mast cell activation in vitro. As initial approaches to this, members of the Section are measuring specific cytokine production by mast cells activated through the IgE receptor or via exposure to specific chemokines and cytokines.

A particularly important advance in this area has been the development of a system to transfect mast cells with exogenous genes. For example the chemokine TCA-3 is expressed by stimulated mast cells. In a set of studies the promoter for TCA-3, and progressively deleted derivatives of it, are linked to a CAT reporter gene and transfected into mast cells in culture. The normal TCA-3 upstream element responds to stimulation and a minimal promoter element has been assigned to the first 82 bases. Additional regulatory elements are being recognized further upstream of this.

A traditionally powerful focus of this group has been its characterization studies of systemic mast cell disorders, ones which have led to refined classification schemes and new therapeutic insights. To date over 150 affected adults and children have been studied. Four (4) major efforts have been made by Dr. Metcalfe and his colleagues in FY93.

First, they have shown the value of bone scanning in evaluation and follow-up of patients with systemic mast cell disease and recognize that bony disease is a major morbid complication of mastocytosis. Palliative radiotherapy to involved bone sites has been beneficial to patients with severe, narcotic-dependent bone pain.

Second, a new sensitive plasma assay for circulating mast cell tryptase has been evaluated for its diagnostic utility.

Third, investigators are pursuing a recent anecdotal report suggesting that recombinant alpha interferon leads to partial remissions of mastercytosis. Three (3) patients have been treated on this protocol with encouraging results.

Finally, a demonstration in vitro that stem cell factor promotes mast cell growth in differentiation provides one logical focus for studies of dysregulated growth in mastocytosis. It is reasonable that stem cell factor or other stimulatory cytokines could be present in abnormally high levels in the tissue of mastercytosis patients. The investigators are exploiting the skin blister model to induce the accumulation cytokines in the skin of normal subjects and mastocytosis patients. Levels of stem cell factor itself as well as TNF-alpha, IL-4, IL-5, and GM-CSF are being measured.

The Mast Cell Physiology Section has also sustained for several years a productive series of studies of food allergy, some in collaboration with Professor P.V. SubbaRao of Bangalore, India. As part of this effort the team has isolated and purified a major shrimp allergen and shown it to be tropomyosine. Two specific tropomyosine peptides appear responsible for allergy to these and related crustaceaems in humans. The finding also permits development of novel, purified, and specific antigens for epicutaneous testing.

The **Bacterial Pathogenesis Unit** is headed by Dr. Thomas Russo. During the past year Dr. Russo and his colleagues have worked in 3 project areas, all dealing with important biological questions of E.coli. In their first and most longstanding project the investigators are evaluating a family of capsule-minus E.coli strains that they generated by transposon mutagenesis. In past years these strains were characterized biologically and shown to be resistant to killing by complement. In all, 17 independent mutants were created; 8 proved to have no capsule. These capsule-deficient strains were proven to be isogenic and involved genes linked to 3 different genomic Xba-I fragments. Thus far, it is known that at least 5 different genes have been disrupted and, by sequencing one resembles a capsule transport gene found in other pathogenic gram-negative bacteria.
A major focus of the past year's work has been the assessment of the biological implications of capsule deficiency by testing the mutants in 3 different animal models. The cumulative data show that higher numbers of these mutants are required to grow readily in animals or kill them. This indicates that E.coli capsular polysaccharide is, of itself, a bacterial virulence factor.

The creation of isogenic capsule-minus mutants provided the investigators important tools for studying the overall regulation and expression of E.coli capsule. As it turns out, the E.coli strain normally makes only group 2 capsule. Other bacterial strains generate group 1 capsule. In a series of studies Dr. Russo and his colleagues showed that the strains of E.coli they are working with possess the genes for both group 1 and group 2, although only group 2 is normally produced. They detected the presence of 3 genes in these bacteria that are typically necessary for regulation of group 1 capsule. By specifically mutating each of these 3 capsule regulatory genes it was possible to uncover the presence of additional regulatory genes and to assign other positive and negative regulatory actions on group 2 capsule of each of these genes. Some of the genes which positively regulate group 1 capsule are negative regulators of group 2 capsule. The bacteria possess critical elements that define which capsule it will express, perhaps depending upon environmental pressures that play upon it. The expression of group 2 capsule in various human infections provides recognition for the first time of the genes which regulate expression of that capsule type yielding new insights into E.coli biology.

In a third project Dr. Russo is collaborating with Dr. Walter Stamm of the University of Washington. For many years Dr. Stamm has been a leader in studying the problem of recurrent urinary tract infections in women. A standing debate involves the origin of the bacterial strains associated the recurrent infections. The most cited data imply that women with recurrent urinary tract infections are repeatedly infected with new bacterial strains.

Using the sensitive technique of pulsed field gel electrophoresis Dr. Russo is able to define the specific "fingerprints" of discrete bacterial strains. He applied this technique to DNA recovered from several dozen bacterial isolates from 20 women. The initial data are quite compelling that 2/3 to 5/6 of all episodes of recurrent urinary tract infection in these women is due to reinfection with the identical bacterial strain indicating that a reservoir exist for the persistence of the bacteria, presumably in the lower GI tract.

These findings suggest a number of useful clinical studies to better understand the ecology of E.coli in woman with recurrent infection and for defining new suppressive approaches to infections.

The **Clinical Mycology Section** is headed by Dr. John Bennett. Dr. K.J. Kwon-Chung is also a tenured senior investigator in the Section. Both Dr. Bennett and Dr. Kwon-Chung direct a number of research projects; these are well integrated in the Section with collaborations among the investigators for most of them. With Dr. Antonia Geber and Dr. Peter Williamson, Dr. Bennett and Dr. Kwon-Chung are undertaking a major study of Candidaalbicans and the disease it produces. One of these efforts entails a detailed molecular analysis of the fungal-maltase gene. Both the cDNA and genomic clones of the gene have been isolated from C-albicans and the upstream binding sequences have been identified and sequenced as well. It has been possible to take the putative promoter region of the maltase gene and insert it upstream of a reporter gene encoding luciferase. This construct was then transfected into C-albicans and the promoter activity was measured with a fluorimeter. Maltase expression is inducible, as was the level of luciferase expressed from the isolated promoter, but the levels have still been too variable. Several means of stabilizing luciferase and of improving this transient expression assay system are being worked on. In related studies, 2 forms of the maltase gene have been purified and their structural and functional relationships have been defined.

A major new feature of this project is an investigation of the molecular basis for Candida species resistance to azole antifungal drugs. One likely site for mutations leading to azole resistance is the 5, 6 desaturase gene. This has been cloned from 2 different Candida species. Over the coming 2 years, with substantial gift funding from Pfizer, Inc. it is intended that this gene be studied in detail to determine whether mutations can be identified within it and clinical isolates that are azole resistant.

About 3 years ago members of the Section undertook a multi-center collaborative trial to determine whether fluconazole is effective for candidemia. A former fellow in the Section, Dr. John Rex, is the principal investigator of the study and remains its director since moving to the University of Texas in Houston. The study has now accrued the desired numbers of patients and the data are being assessed.

Dr. Peter Williamson is conducting biochemical and molecular studies of the important pathogenic fungus Cryptococcus neoformans. It is already known that melanin is a virulence factor for this yeast. To understand this process the diphenol oxidase has been purified and the gene encoding it has been cloned. Efforts will be made to specifically delete the gene from Cryptococcus neoformans and verify that its absence leads to reduced virulence of the yeast for mice.

A major wish of this Section is to develop a vaccine for cryptococcosis. One approach being utilized involves ongoing studies of surface proteins of Cryptococcus neoformans. By now 3 surface proteins have been isolated and partial amino acid sequences for each has been obtained. An oligonucleotide probe based upon the sequence for one of the proteins successfully identifies a single fragment in a DNA digest of C neoformans. It is hoped that the gene itself and the genes for the other 2 proteins will be identified in the coming year.

In a related but more advanced manner a cryptococcal vaccine is already under study by members of the Section in collaboration with scientist in NICHD who developed the reagent. For the past 2 years clinical studies have been undertaken in NIAID of a purified type A cryptococcal capsular polysaccharide linked to tetanus toxoid. In initial studies no known individuals were shown to develop a substantial antibody response to the antigen and serum from vaccine recipients with protectives from mice upon subsequent challenge with lethal numbers of organisms.

Within the past year the investigators initiated and are half completed with a second vaccine study of 90 individuals infected with HIV. Volunteers are being assigned to either of 3 groups according to their CD4 count. It is hoped that antibody rises will be observed in response to vaccination, at least in individuals with relatively near normal CD4 counts. Should this be the case, the investigators propose that the NIH conduct large efficacy field trials.

Dr. Kwon-Chung personally directs 2 major projects. For twenty years she has been undertaking basic investigations of Cryptococcus neoformans. A major achievement of the past year has been the cloning of a gene responsible for the formation of the extracellular capsule of Cryptococcus neoformans. It is apparent from older detailed studies of acapsular mutants of cryptococcus that there are a number of genes potentially involved. The gene encoding one protein responsible for capsule (CAP67) was successfully cloned and detailed study shows mutant 59 to have a lesion in the same genetic locus. In the coming years efforts will be undertaken to characterize the product of gene 67 and to identify and characterize the other genes responsible for capsule formation in cryptococcus.

A second series of molecular investigations has led to the development of specific DNA probe sequences that will discriminate genetically related strains of Cryptocuccus neoformans from unrelated strains. This has already been utilized in molecular epidemiologic studies to define the derivation and relatedness of a large number of strains isolated in the wild and from people world-wide.

Another series of studies is attempting to investigate virulence factors in Aspergillus species. To this end the Aspergillus fumigatus hydrophobine gene has been identified and cloned. Hydrophobine is important for the full maturation and release of virulent spores by Aspergillus fumigatus. Mutants in the gene lead to incomplete maturation and release of the spores and a greater ability for them to be phagocytosed and killed by neutrophils. Dr. Yun Chang, with the assistance of Dr. Mark Parta, is creating isogenic strains of Aspergillus fumigatus that contain an intact hydrophobine gene and others in which it is disrupted by homologous recombination of a DNA fragment bearing a selectable marker. The hope is to determine the extent to which the virulence of the organism is diminished by this specific genetic lesion. A success in this area would lead to entirely new avenues for therapeutic interventions.

FY 93 witnessed the completion of a long series of productive projects headed by Dr. Michael Kaliner and members of the **Allergic Diseases Section**. A substantial effort was maintained in the most long standing of these protocols whose intent was to define the cellular, biochemical, and molecular features of inflammatory responses of tissues after exposure to allergens. The first part of this project consisted of continuing characterization of the cells infiltrating inflamed measle mucosa in allergic individuals. Human nasal inferior turbinates were found to contain infiltrating lymphocytes of which the proportion that are CD4+ and CD8+ are similar to that circulating in a blood stream. Mast cells, immunoglobulin-secreting and plasma cells were found in the mucosa. IgA-positive cell predominated with no IgE-positive cells. The infiltrating mast cells were positive for IgE.

A quantitative PCR technique was established to measure levels of messenger RNA in human nasal mucosa for IL-2, IL-4, and gamma interferon. The assay is currently working and the levels are being defined.

A third part of this project involved development of a new fluorescent assay for the proportion of lymphocytes expressing cytokines using a direct FACS analysis. Thus far, the technique set up by Dr. Prussin has been standardized for circulating blood cells. It gives good agreement with other assay methods.

In the final component of this project Dr. Chowdhury is collaborating with Dr. Claire Fraser of the Institute for Genomic Research on cloning and expressing the human histamine H-1 receptor. In 1992 the Bovime receptor was cloned. Because of the pivotal role that the histamine receptor plays in allergic disorders this project was given a high priority. Dr. Chowdhury has succeeded in cloning a gene from a human library that is all of the appropriate features expected for a human H-1 receptor including high levels of conserved sequence with the Bovime receptor. The intent over the coming year is to sustain this project through the Office of the Chief to raise antisera to predicted peptides and, to develop assays for H-1 receptor expression, to completing cloning and sequencing of the gene, and to express the protein in cells.

Except for the above cloning project, the remainder of this project is terminated. A second project that is being concluded is a large series of studies of nasal responses to allergines. This has bee a very productive project leading to numerous publications which in recent years have concentrated on the inflammatory mediators, neuropeptides, and other protein constituents of mucus.

A model system has been established and supervised by Dr. Martha White in which nasal secretions are provoked by instilling substances into the nose. The secretions are then collected and their constituent contents measured. Among the peptides measured in the past year are substance P and the calcitonin gene-related peptide, the coholineogic vasoactive intestinal peptide, neutral endopeptidase and secretory lukoprotease inhibitor.

A small project has been maintained for many years as a collaborative effort between Dr. Kaliner and Dr. James Shelhamer of the Critical Care Medicine Department. Investigators recently reported that human nasal mucosa contain endothelin-1. ET-1, as it is abbreviated, is a sensory neuropeptide with potentially important functions. It is secreted from the glans and would appear to participate in regulating the overall glandular responses. A second part of this project confirmed that the muscarinic N3 receptor is primarily involved in the cholinergically-regulated component of nasal glandular secretions.

Over the past year the **Mucosal Immunity Section**, under the direction of Dr. Warren Strober, sustained vigorous efforts in 4 project areas. The first maintains the long standing interest of the Section in Inflammatory Bowel disease and the immunologic contributions to it. It is presumed that inflammatory bowel diseases are due to a dysregulation of the immune system, perhaps in response to the constant presence of a wide variety of mucosal antigens.

Most recently the investigators working on this project have concentrated on comparisons of T lymphocytes purified from the lamina propria of human bowel and T-cells purified from the circulation of the same individuals. It was apparent by several criteria that these cells differed. Additional differences were noted when comparing the responsiveness of the lamina propria T-cells from normal individuals and from individuals with either ulcerative colitis or Crohan's disease. In a normal individual the proliferative unresponsiveness of lamina propria T-cells was in marked contrast to the great capacity of these cells to release lymphokines on stimulated via accessory pathways. Lamina propria T-cells from patients with inflammatory bowel disease were even more unresponsive to routine stimuli but reduced secretion of some lymphokines, in patterns that differed for different forms of the inflammatory bowel disease.

Although the specific reasons for these differences will remain the subject for further studies the work has already shown important features of immune regulation in the normal gut and in the inflamed gut. Lymphocytes in the normal gut may show unresponsiveness as a protective measure so that antigen induced apoptosas is prevented.

The second project area is directed at understanding the cellular and molecular processes which permit and lead to differentiation of B lymphocytes that secrete IgA. The most noteworthy accomplishment of the past year was a demonstration by a variety of sophisticated techniques that the switching of cells to release the IgA isotype is associated with splicing together RNA transcripts from widely separated segments of the gene coding for immunoglobulin, a process termed "Transplicing".

To accomplish this, the investigators had already developed a model system in which cells can be induced in fair number to express IgA. Carefully controlled RT-PCR experiments using various pairs of primers spanning wide regions of the genome showed that RNA products from widely seperated genes were synthesized. The transplicing process incorporates transcripts encoded from the immunoglobulin Iα region. The presence of that product in the transpliced message provides a target for further studies to determine whether means of augmenting or reducing Iα transcription raises or lowers rates of IgA switch.

In a related project area the effects of various regulatory lymphokines are measured on the expression by human lymphocites of the individual IgG subclasses. These studies showed that subclass-specific transcription falls into two patterns: expression of the germline transcript leading to subclasses 1 and 3 is induced by proliferative stimulation of the cells in the presence of Il-2 and/or Il-4; while transcription leading to subclasses 2 and 4 is induced by Il-4 and gamma interferon without need for a proliferative stimulus.

IL10 is a recently recognized cytokine that possesses potent suppressive properties. The possible role of IL10 in the inflammatory cascade was explored by measuring rates of IL10 RNA synthesis in response to a panel of inflammatory cytokines. Of 7 cytokines tested only TNF-α induced IL10 RNA substantially. Specifically, TNF-α led to an 80 to 120 fold increase in IL10 message production while the others cytokines barely achieved a 3 fold induction. The induction by TNF-α was dose dependant, took several hours to evolve, and involved upregulation of message synthesis.

This observation has particularly important relevance to the inflammatory process. It is known that multiple cytokines are released but in a normal regulated event the inflammatory cytokine production is only temporary, turning towards normal after several hours. As a major inflammatory cytokine, TNF-α has the capacity to produce IL10 which, in turn, is able to shut off the synthesis of the inflammatory cytokines. This provides a negative feedback system to appropriately regulate the inflammatory response.

Longstanding clinical and basic research interest in this Section have revolved about a variety of primary immunodeficiency disorders particularly common variable immunodeficiency. In the past year members of the Section extended earlier studies of immunoregulatory defects in patients with common variable immunodeficiency. Prior work for the group had shown that T-cell from some patients with common variable immunodeficiency fail to release normal amounts of Il-2 in response to proliferative stimuli. If the cells are stimulated, however, through accessory pathways normal amounts of cytokine can be detected. Similarly B-cells from patients with common variable immunodeficiency show a defect in isotype switch. In vitro stimulation of cells purified from these patients with anti-CD40 antibody and IL10 improved isotype production. This series of experiments suggest that the failure of B-cells from common variable immunodeficiency patients to function is reversible, implying that a variety of focused interventions could correct the immunodeficiency disorder.

1992-1993
Honors and Awards
Laboratory of Clinical Investigation, NIAID

During FY93 several honors were bestowed on LCI staff members.

Dr. Michael Kaliner -

Recipient of the National Asthma Award for Biomedical Research, from the First National Conference on Asthma Management.

Delivered the Stephen D. Lockey, Sr., M.D. Memorial Lecture, Pennsylvania Allergy Society.

Delivered the Phillip Pratt, M.D. Distinguished Lectureship, University of North Carolina

Served as a Visiting Pulmonary Scholar, Consortium of North Carolina Programs.

Dr. Dean Metcalfe -

Elected, Chairman, Training Program Directors, AAAI.

Director, American Board of Allergy and Immunology.

Dr. John Bennett -

Delivered the Bowman Lecture of the University of Virginia on October 22, 1992.

Delivered the Maxwell Finland Lecture of the Infectious Diseases Society of America on October 11, 1992.

Dr. Stephen Straus -

Appointed Associate Editor of Virology and Field's Virology.

Appointed to NIH Recombinant DNA Advisory Committee and the AIDS Data Safety and Monitoring Board.

Dr. Menachem Rottem -

Recipient of the First Wegener's Foundation Research Award for a post-doctoral fellow.

Janet Dale, RN -

Recipient of NIH Directors Award

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

A01 AI 00057-20 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic Studies on Pathogens Causing Cryptococcosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: K.J. Kwon-Chung, Research Microbiologist, Clinical Mycology Section, LCI, NIAID
Others: Yun C. Chang, Staff Fellow, Clinical Cycology Section, LCI, NIAID
Ashok Varma, Microbiologist, Clinical Mycology Section, LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cloning of a gene responsible for the formation of extracellular polysaccharide capsule in *Cryptococcus neoformans*, a major opportunistic fungal pathogen causing infection primarily in immunocompromised patients: The extracellular polysaccharide capsule of *C.neoformans* has been identified as the major vrulence factor of the organism. A wild type genomic DNA library of *C. neoformans* B-3501 was used to complement an *ura5* acapsular mutant(B-4131,*cap 67*). The library was constructed in a plasmid vector containing the *URA5* gene as a selection marker. After electroporation, a plasmid containing a 4.5kb DNA insert was rescued in *E.coli* from one of the Cap$^+$ transformants. By using overlapping subclones of the 4.5kb DNA fragment, the minimum region required for complementation of B-4131 Cap$^-$phenotype was identified on a 2.9kb fragment. The transformants became acapsular when they lost their transforming plasmids during growth on non-selection media. In addition, the 2.9kb fragment isolated from the wild type strain was able to restore the Cap$^+$phenotype in another acapsular mutant(*cap 59*). The *CAP 59* was one of the five capsule-associated loci which were previously mapped. These results indicated that we cloned the gene *CAP 67*.

A genomic probe for strain identification in *C.neoformans* was constructed for epidemiological study: A plasmid, UT-4, containing *URA 5* gene with 3kb flanking sequences and telomeric sequence of *C. neoformans* was found to be a useful probe for DNA finger printing. The probe was specially useful for strain identification of the *gattii* variety of *C. neoformans*. Using the probe, we could identify that sevaral strains of *C.neoformans* var.*gattii*

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-A1 0058-19 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis and Chemotherapy of Herpesvirus Infection in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus - Senior Investigator - LCI, NIAID
Others: J. Cohen - Senior Staff Fellow - LCI, NIAID
R. McKenzie - Medical Officer - LCI, NIAID
R. Kost - Medical Staff Fellow - LCI, NIAID
T. Heineman - Medical Staff Fellow - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI, NIAID
J. Dale - Research Nurse - LCI, NIAID
J. Lekstrom - Medical Staff Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*

J. Rooney, Senior Staff Fellow, LOM, NIDR; A. Notkins, Chief, LOM, NIDR; L. Stanberry, University of Cinncinnati; P. Krause, CBER/FTA; R. Hunziker, Transgenic Mouse Facility

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.4	4.5	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to characterize the pathogenesis , natural history and therapy of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. Our clinical emphasis has been on oral and genital herpes and zoster in the normal and the immuno-compromised host. Over the years we established the value, long term efficacy and safety of oral acyclovir for suppression of recurrent genital herpes and more recently oral herpes as well. During the past year we conducted phase I and II studies of topical capsaicin, a substance P depleting agent and determined that, as tolerated, it does not inhibit virus reactivation. We also initiated collaborative studies of BVaraU, a new drug for zoster. Also in the past year, we identified and studied the first known instance of a persistent acyclovir-resistant HSV2 infection in an immunologically normal individual.

The major basic research thrust of this laboratory has been to define molecular aspects of HSV and VZV latency and pathogenesis. We are examining the role of the HSV I and II latency-associated transcripts (LAT) in control of virus latency and reactivation. Recombinant viruses deleted for LAT expression are being studied *in vitro* and in animal models. We have begun to create transgenic mice expressing HSV genes neighboring and/or including the LAT gene. We are also studying specific LAT promotor elements.

Work on VZV latency and gene regulation has concentrated on genes 4, 10, 28, 21, 61, 62, and 63. Because gene 29 is expressed in latency and 28 is not, we are studying the regulation of these two genes. We found that they share a common promoter domain with a unique cis-acting element that coordinates regulation and expression of both genes.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00154-17 LCI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Immediate Events in Hypersensitivity

PRINCIPAL INVESTIGATOR

P.I.:	M. A. Kaliner	Head, Allergic Diseases Section	LCI/NIAID
Others:	M. V. White	Senior Staff Fellow	LCI/NIAID
	A. Daniels	Biologist	LCI/NIAID
	B. Chowdhury	Medical Staff Fellow	LCI/NIAID
	H. Nagata	Fogarty Fellow	LCI/NIAID
	Calman Prussin	Medical Staff Fellow	LCI/NIAID
	Tetsuo Ikezono	Fogarty Fellow	LCI/NIAID

J. Baraniuk, Georgetown University; J. Goff, Georgetown University (Contract #NO-1-AI-22665); C. Fraser, The Institute for Genomic Research

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	4.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

This project has focused on the allergic inflammatory response seen in tissues after allergen exposure. In the first part of the project, the identification of the cells found in the human nasal mucosa were carefully measured, and then the effects of allergen exposure were examined. The resident cells consisted of lymphocytes, mast cells, and plasma cells. After allergen exposure, the major changes were activation of the lymphocyte (increased expression of the IL-2 receptor), an increase in eosinophil infiltration, and degranulation of mast cells.

The bulk of the time on this project this year was spent developing techniques to measure cytokine expression quantitatively, to assess lymphocyte phenotypes by FACS analysis, and to clone the human H-1 receptor.

Project Terminated 1992 - 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00249-12 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section, LCI/NIAID
Others:	Peter Bianchine, M.D.	Medical Staff Fellow, LCI/NIAID
	Menachem Rottem, M.D.	Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

Laboratory of Host Defense Mechanisms, NIAID Dr. John Gallin

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL	OTHER
2.00	1.50	0.50

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Local radiotherapy was successful in decreasing refractory bone pain in mastocytosis patients with significant bone erosion. This severe intractable bone pain is seen in patients with aggressive disease and is usually treated unsuccessfully with narcotic analgesics.

Studies are underway to assess cytokine profiles in mastocytosis patients. Suction blisters are raised over urticaria pigmentosa lesions which contain collections of mast cells. Blister fluid is assayed for cytokines appearing in blister fluid over several hours.

A new improved serum assay for mast cell-derived tryptase may result in improved diagnosis for systemic mast cell disorders.

Significant liver disease is seen in association with aggressive mast cell disease (Categories II and III). Pathology includes mast cell infiltrates, inflammation around the portal triads, and fibrosis.

A retrospective review of the bone scans of 75 mastocytosis patients has demonstrated that bone scanning provides an efficient, noninvasive method of assessing and following active bone and bone marrow involvement. As the disease progresses, abnormalities on bone scan become increasingly diffuse. Progression of disease on serial bone scans occurs in patients more likely to have a malignant course.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00279-11 LCI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR M. A. Kaliner Head, Allergic Diseases Section LCI/NIAID

Others:

COOPERATING UNITS *(If any)*
J. H. Shelhamer, M.D., C. Logun, M.D. (Critical Care Medicine, Clinical Center, NIH)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human and feline bronchial and nasal mucosa have been developed in order to examine the controls of mucus secretion. Two projects were completed. The first defined the distribution and actions of endothelin in the human nasal mucosa, and the second confirmed that the muscarinic M3 receptor is primarily involved in cholinergic regulation of nasal glandular secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOA AI 00354-11 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Warren Strober, M.D.	Chief, MIS Section	LCI, NIAID
Monica Boirivant, M.D.	Special Volunteer	Fogarty Fellow

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The working hypothesis guiding our research on the nature of inflammatory bowel diseases (IBD) is that these diseases, particularly Crohn's disease, are due to abnormal regulation of responses to ubiquitous mucosal antigens. In recent years, this hypothesis has resolved itself into studies on the ability of T cells obtained from IBD patients to produce immunoregulatory lymphokines under various conditions. During the current period we have first defined the capacity of control T cells derived from lamina propria and the peripheral blood (LP and PB T cells, respectively) to undergo proliferation and to produce lymphokines when stimulated via defined T cell activation pathways. We found that when stimulated via the TCR/CD3 pathway (with several different CD3 and TCR antibodies) LP T cells exhibited 10-fold less proliferation than PB cells; on the other hand, when stimulated via the CD2 accessory pathway, LP T cells exhibited considerably more normal proliferative responses (as compared to PB T cells). This non-responsiveness was probably a form of peripheral T cell anergy, since partial recovery of cell function was obtained by pre-incubation of T cells with IL-2 (in the absence of TCR/CD3 stimulation). In contrast to this "proliferative unresponsiveness", LP T cells exhibit a greatly heightened capacity to produce lymphokines, including IL-2, IFN-γ and IL-4. In this regard, while LP T cells secreted 5-10-fold more IL-2 than PB T cells under all stimulatory conditions, they secreted remarkable amounts of IL-2 (>30,000 pg/ml/10^5 cells) when stimulated via accessory pathways. In comparable studies of IBD lamina propria T cells, several important differences were noted. Firstly, while responses induced via the TCR/CD3 pathway were 5-fold lower than the already reduced control LP T cell responses, responses induced via accessory pathways (CD2 and CD28) were preserved. Secondly, T cells from ulcerative colitis patients, stimulated via accessory pathways, manifested somewhat reduced IL-2 and IL-4 secretion (but not IFN-γ secretion). Thirdly, T cells from Crohn's disease patients stimulated in a similar fashion manifested reduced IL-2 secretion, but vastly increased IFN-γ and IL-4 secretion (5-10-fold increases). These studies therefore define a basic lymphokine secretory defect associated with Crohn's disease.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00 356-11 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Regulation of IgA Immunoglobulin Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Warren Strober, M.D.	Chief, Mucosal Immunity Section, LCI, NIAID
Other:	Yoshio Wakatsuki, M.D.	Visiting Fellow, MIS, LCI, NIAID
	Rolf Ehrhardt, M.D.	Visiting Fellow, MIS, LCI, NIAID
	Markus Neurath, M.D.	Visiting Fellow, MIS, LCI, NIAID
	Milan Basta, M.D.	Visiting Associate, MIS, LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is focused on the cellular and molecular processes that occur during the differentiation of IgA B cells, the main type of B cell developing in mucosal lymphoid follicles. During this phase of our studies we continued our examination of the role of germline transcripts in isotype differentiation generally and IgA switching specifically. In particular, we gathered several pieces of evidence that isotype switching is associated with "transplicing", i.e., splicing of RNA transcribed from widely separated segments of the Ig gene. First, we demonstrated that sIgA+ B cell lymphoma cells (CH12.LX.A2 cells) which lack Iα gene segments and are transfected with plasmid constructs that can be induced to express Iα, produce Iα-Cα (germline) transcripts. Since it is unlikely that the transfected Iα gene integrated at a site close to Cα in all cells, this result strongly suggests that Iα is transpliced to Cα in the transfected A2 B cells. Next, we showed that CH12.LX B cells, cells that constitutively produce Iα germline transcripts and spontaneously switch from sIgM+ to sIgA+ B cells, produce J_H-Iα transpliced mRNA transcripts. The latter were shown with highly controlled RT-PCR techniques, followed by sequencing of the amplified cDNA. Finally, we showed that dexamethasone, an agent that induces sIgM+ CH12.LX B cells to differentiate into sIgM+/sIgA+ switch intermediate cells, also induces increased levels of J_{H2}-Iα transpliced mRNA. Taken together, these data strongly suggest that CH12.LX B cells undergoing isotype switching pass through a stage during which they transplice a VDJ-Iα-Cα message that is further processed to a VDJ-Cα message prior to switch rearrangement. Such transpliced messages may "direct" subsequent DNA rearrangement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 0429-08 LCI Project Terminated

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Studies on Nasal Responses

PRINCIPAL INVESTIGATOR	M. A. Kaliner	Head, Allergic Diseases Section,	LCI/NIAID
Others:	M. V. White	Senior Staff Fellow	LCI/NIAID
	Y. Igarashi	Fogarty Fellow	LCI/NIAID
	C. H. Lee	Fogarty Fellow	LCI/NIAID
	B. Mosimann	Special Volunteer	LCI/NIAID
	R. Moss	Medical Staff Fellow	LCI/NIAID
	T. Scott	Medical Staff Fellow	LCI/NIAID
	A. Daniel	Biologist	LCI/NIAID

COOPERATING UNITS *(if any)*
J. H. Shelhamer, M.D. (Critical Care Medicine, Clinical Center, NIH);
R. Lebovics, M.D. (NIDCD, NIH)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.7	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The nasal mucosa is the initial site for detoxifying airborne pathogens, irritants, allergens, and toxins. Analysis of nasal secretions has provided important insights into the mechanisms by which mucous membranes respond to these encounters. In secretions, there are a variety of proteins playing roles in protecting the underlying epithelium. For instance, neutral endopeptidase (NEP) is secreted in order to metabolize neuropeptides which might otherwise stimulate the epithelium. Secretory leucoprotease inhibitor (SLPI) is a major secretory protein which acts to prevent neutrophil elastase injury to the epithelium.

Provocation of allergic individuals with allergen led to the secretion of three neuropeptides (SP, CGRP, and VIP), despite the presence of NEP. Aspirin challenge of asthmatics led to the secretion of glandular products in response to dramatically increased production of leukotrienes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

AI 00430-09 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Varicella-Zoster Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jeffery I. Cohen, Senior Staff Fellow, LCI,NIAID
Others: S. Straus, Chief, LCI, NIAID
J. Meier, Clinical Associate, LCI, NIAID
R. Kost, Clinical Associate, LCI, NIAID
H. Moriuchi, Fogarty Fellow, LCI, NIAID
K.E. Seidel, Bio. Lab. Tech., LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.3	PROFESSIONAL: 3.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primary infection with varicella-zoster virus (VZV) causes chickenpox, and reactivation of the virus from latency results in zoster. The goals of this project are to identify and determine the function of VZV genes that are expressed during active infection and during latency in the body.

VZV genes that are expressed during active infection and regulate viral gene expression in vitro are being studied. Analysis of VZV gene products during acute infection indicate that at least four VZV genes, ORF4, ORF10, ORF61, and ORF62 transactivate the expression of other VZV genes in vitro, and two of these genes (ORF10, ORF62) activate transcription directly. Thus, a limited number of VZV gene products are critical during acute viral infection in regulating VZV replication. Three of these VZV genes (ORF10, ORF61, and ORF62) can complement the function of their herpes simplex virus homologs. Analysis of trigeminal ganglia from human cadavers without active evidence of VZV infection indicates that at least two VZV genes, ORF29 and ORF62, are expressed during viral latency. These genes are expressed exclusively in non-neuronal (satellite) cells. In contrast, other VZV genes, ORF10, ORF28, and ORF61, are not expressed during latency. Therefore, the mechanism of VZV latency in the central nervous system of humans is markedly different from that of the most closely related human herpesvirus, herpes simplex virus.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00432-09 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of immune responses in humans and non-human primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID

Other: Monica Boirivant, M.D., Visiting Fellow, MIS/LCI/NIAID
Atsushi Kitani, M.D., Visiting Fellow, MIS/LCI/NIAID
Chingchai Wanidworanun, M.D., Visiting Associate, MIS/LCI/NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

I) To further elucidate B cell isotype differentiation in humans we defined the conditions necessary for IgG sub-class-specific germline transcript (GLT) production in human peripheral blood cells. We found that induction of GLT for the various IgG subclasses fell into two patterns. Induction of Cγ3 and Cγ1 GLT, transcripts of genes in the first human duplication unit, generally required a proliferative stimulus (SAC) and was brought about by SAC plus IL-4 (Cγ3 GLT) and SAC alone or SAC plus IL-2 (Cγ1 GLT); in contrast, induction of Cγ2 and Cγ4 GLT, transcripts of genes in the second duplication unit, was induced accomplished with cytokines alone: IFN-γ(Cγ2 GLT) and IL-4(Cγ4 GLT), and was not augmented by addition of a proliferative stimulus. Finally, we found that IFN-γ inhibited IL-4-induced Cγ3 and Cγ4 GLT (with or without SAC). These findings establish that the various human IgG subclasses manifest distinct requirements for the regulation of early steps in isotype differentiation. In addition, they suggest that human Cγ genes exhibit patterns of regulation related to their respective gene duplication units.

II) IL-10 has recently been shown to have an important influence on human B cell differentiation. To learn about the regulation of this cytokine we tested the hypothesis that its production by monocytes is under control of one of the inflammatory cytokines. In initial studies, we co-cultured purified human peripheral blood monocytes with a panel of cytokines including TNF-α, IL-1α, IL1-β, IL-6, GM-CSF, TGF-β and IFN-α, and then measured IL-10 mRNA production using a semi-quantitive reverse transcription-polymerase chain reaction (RT-PCR) technique. We found that TNF-α has a major effect on IL-10 mRNA production, inducing an 80-120-fold increase over baseline; in contrast, none of the other cytokines have more than a 2-3-fold effect. In later studies, we showed that the induction of IL-10 mRNA by TNF-α is dose-dependent and begins between 8-24 hr following the addition of TNF-α; this suggests that the increased IL-10 mRNA level is due to *de novo* mRNA synthesis rather than mRNA stabilization. Taken together, these results suggest that TNF-α plays a key role in the induction of IL-10 in human monocytes; as such, it induces a molecule that provides negative feedback to its own production. In addition, these studies suggest that TNF-α, via its effect on IL-10, may affect overall levels of B cell proliferation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00470-08 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chronic Epstein Barr Virus Infection and Chronic Fatigue Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus - Senior Investigator - LCI, NIAID
J. Dale - Clinical Research Nurse - LCI, NIAID
E. Benson-Grigg - Guest Research Nurse - LCI, NIAID
R. McKenzie - Medical Officer - LCI, NIAID
J. Cohen - Senior Staff Fellow - LCI, NIAID
A. O'Fallon - Clinical Research Nurse - LCI, NIAID
W. Strober - Senior Investigator - LCI, NIAID
M. Sneller - Medical Officer - LIR , NIAID

COOPERATING UNITS *(if any)*

J. Grafman (LNM, NINCDS), H. Rotbart (Univ. of Colorado), M. Demitrack (Univ. of Michigan), M. Altemus, P. Gold (NIMH), P. Peeke, D. Garcia, G. Chrousos (NICHD), Scott Fritz (PRI, FCRF)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	3.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to characterize severe chronic infections with Epstein Barr Virus (EBV) and to elucidate multiple aspects of the chronic fatigue syndrome which was, earlier, considered to be related to EBV infection. To date this research project has involved about 230 patients. Included are 7 patients who were diagnosed with severe chronic EBV infections on the basis of clinical, histological, molecular and serologic features. We continue to examine immunologic features of patients with severe chronic EBV-associated lymphoproliferation and explore treatments. Acyclovir, alpha and gamma interferons proved of little value, but immunosuppressive therapies are being used with good long-term results.

Detailed immunologic, neurologic, endocrinologic and psychologic studies are being conducted on selected patients with chronic fatigue. To date, we still have no consistent laboratory abnormality that permits a clear diagnosis of the chronic fatigue syndrome, however, we have been pursuing the basis and meaning of group abnormalities in neuropsychiatric, immune and endocrine systems. A series of studies of the pituitary-adrenal responsiveness to corticotropin releasing hormone and ACTH and of neuropeptide and catechol levels in spinal fluid suggest deficient central CRH release. Since CRH induces CNS arousal, these neuroendocrine findings suggest a new mechanism whereby the lethargy of Chronic Fatigue Syndrome patients may be explained. We are pursuing these observations in a new series of studies and a fresh patient cohort. Arginine-Vasopressin infusions have been given to 24 patients and 20 controls to stimulate and test the HPA axis. The results will be available in early FY 94. Thirty-three patients were enrolled already in a placebo-controlled trial of hydrocortisone treatment. It should permit us to test the hypothesis that corticosteroid deficit leads to symptoms. We also recognized discrete abnormalities in CFS patient lymphocyte phenotype and in vitro responsiveness to mitogens in patterns suggesting mild immune activation. There is a reduction in naive (CD4xCD45RA) T cells and an increase in memory (CD4xCD45RO) T cells bearing adhesion markers (LFA3, CD29, etc.). We have begun to pursue these findings in several other laboratory studies involving purified cell populations from patients and controls. A vigorous, multidisciplinary approach to the syndrome continues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00495-06 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Events in Mast Cell Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Other:	Robert J. Hohman, Ph.D	Expert	LCI/NIAID
	Thomas Hultsch, M.D.	Fogarty Associate	LCI/NIAD
	Raynaldo A. Martin	Biologist	LCI/NIAD
	Susan A. Bardwell	Howard Hughes Fellow	LCI/NIAD

COOPERATING UNITS *(if any)*

Dr. Sue Goo Rhee, Lab. of Biochem., Sect. on Sig. Transduction, Bethesda, MD
Dr. Alam Weissman, NCI

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biochemistry of IgE receptor-mediated activation of mast cells is being studied in two separate but complementary projects which are summarized below.

1) Inhibition of IgE receptor-mediated exocytosis from mast cells by the immunosuppressive drugs cyclosporin A (CsA) and FK506.

Immunosuppressive drugs such as CsA and FK506 are considered by many to specifically inhibit T cell functions; however, the same concentration of CsA that inhibits T cell receptor-mediated activation of T cells also inhibits IgE receptor-mediated degranulation of mast cells. These studies have been extended by studying the effect of FK506 and its structural analog, rapamycin, on IgE receptor-mediated degranulation of mast cells. The intracellular target for rapamycin and FK506 (FKBP) is distinct from the intracellular target for CsA (cyclophilin). Like CsA, FK506 inhibits degranulation of mast cells without effecting any other IgE receptor-mediated function; however, FK506 is 100 times more potent than CsA. The IC_{50} for inhibition is 200 nM and 2 nM for CsA and FK506, respectively. Rapamycin and 506BD both compete with FK506 for binding to FKBP. Pretreatment of mast cells with either rapamycin or 506BD prevents the inhibition by FK506. Thus, it appears as through a FKBP-FK506 complex as well as a cyclophilin-CsA complex inhibits receptor-mediated activation of mast cells.

2) Activation of membrane-associated phospholipase C by aggregation of IGE receptors

Currently, little is known concerning the mechanism of activation of PLC and, in fact, it is not even known which of the PLC isozymes present in mast cells is involved in receptor-mediated PI hydrolysis. A Major obstacle to these studies is the lack of a cell-free system in which this activation can be studied. We have established a system in which aggregation of IgE receptors results in activation of membrane-associated PLC. Rat basophilic leukemia (RBL) cells are activated by aggregation of the IgE receptors. The PLC activity in membranes isolated from activated cells is increased by 150% above membranes from control cells. This system will be used to identify which PLC isozyme is activated and the mechanism of this activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00496-06 LCI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interaction Between the Human Immunodeficiency Virus (HIV) and Herpesvirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S. Straus - Senior Investigator - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*
D. Margolis, (University of Massachusetts)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We spent 3 years studying the interactions between HSV and HIV genes at a molecular level. In the first project we examined the mechanism by which HSV-1 activates HIV RNA transcription. The bulk of HSV activation is dependent on the integrity of Sp1 and NF-kB enhancers in the HIV LTR. HSV also activates the LTR by inducing the expression of a cellular protein that binds near the HIV transcriptional start site. Competition experiments proved the binding specificity of the protein and uV-cross linking studies identified a novel 45kD protein. Our former fellow Dr. David Margolis is independently pursuing purification of the protein, termed LBP-2.

In the second series of studies we showed that the HIV tat transactivates the HSV-1 latency-associated gene. Transient expression assays showed marked upregulation of LAT promoter but not other classes of HSV-1 promoters. Site-directed alterations in the LAT promoter sequence defined the general area of the tat target.

This project is being terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00513-06 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Mast Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section, LCI/NIAID
Others: Menachem Rottem, M.D. Medical Staff Fellow, LCI/NIAID
Peter Bianchine, M.D. Medical Staff Fellow, LCI/NIAID
Jaroslaw Dastych, Ph.D. Fogarty Visiting Fellow, LCI/NIAID
Yoseph Mekori, M.D. Fogarty Visiting Scientist, LCI/NIAID

COOPERATING UNITS *(if any)*

Georgetown University (Goff)
Amgen

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	2.50	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mast cells adhere to fibronectin following activation with the mast cell growth factor c-kit ligand (also known as stem cell factor), but not after addition of IL-3. This process is integrin-mediated and follows the specific interaction between c-kit ligand and c-kit. Genistein, an inhibitor of tyrosine kinase, partially prevents c-kit ligand-induced mast cell adhesion. Because c-kit is produced by resident connective tissue cells, it is a strong candidate for the physiologic stimulus responible for the specific tissue localization of mast cells.

Mast cells exhibit spontaneous adhesion to vitronectin. The engagement of mast cells to vitronectin through a vitronectin receptor is followed by an increase in proliferative rate and the rapid phosphorylation of at lease one protein.

IL-3 dependent mast cells undergo apoptosis (programmed cell death) following removal of IL-3. This is prevented by the addition of c-kit ligand, and this effect is not prevented by dexamethasone or cyclosporin. W/W^v mast cells with defective c-kit undergo IL-3 deprivation-induced apoptosis despite the addition of c-kit ligand.

c-Kit ligand in vitro induces mast cell maturation toward the connective tissue mast cell phenotype. Mouse bone marrow-derived mast cells respond to both IL-3 and c-kit ligand and are inhibited by m-CSF, GM-CSF, γ-IFN, and NGF. Mouse peripheral blood mononuclear cell-derived mast cells respond only to c-kit ligand.

Human peripheral blood-derived human mast cells are derived from a $CD34^+$ cell population. CD34 positivity is rapidly lost in culture, while the cell population becomes $Fc_{\epsilon}RI^+$.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00514-06 LCI

PERIOD COVERED

October 1, 1992 to Setember 30, 1993 (To be discontinued September 30, 1993)

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnosis and Treatment of Adverse Reactions to Foods and Additives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section, LCI/NIAID
Others: None

COOPERATING UNITS *(if any)*

Indian Institute of Science, Bangalore, India (P.V. Subba Rao)
Clinical Neuroscience Branch, NIMH (Martin)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
1.00	0.50	0.50

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major shrimp allergen has been isolated and identified as a tropomyocin based on a comparison of amino acid sequences of different peptides generated by proteolysis of the parent molecule. Dot blot and competitive ELISA inhibition using sera of shrimp sensitive patients revealed that antigenic as well as allergenic activities were associated with two peptides. These peptides have been sequenced. These two peptides are similar between cross-reacting Crustaceae, but vary between tropomyosin from phylogenetically distinct species.

Anti-idiotypic antibodies (Ab-2) are the mirror images of idiotypic antibodies (Ab-1). Murine Ab-2 raised by immunizing mice with AB-1 specific for Parthenium hysterophorus functioned as surrogate allergens in in vitro diagnostic tests. Surrogate allergens may be of value in allergen standardization and for in vitro diagnosis particularly when it is difficult to obtain and purify specific allergens in large quantities.

MSG is a GRAS substance used to enhance the flavor of foods. Double-blind placebo controlled challenges of both normal and asthmatic subjects with MSG did not provoke asthma. MSG administered before methacholine challenge did not alter bronchial reactivity to methacholine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 0000548-05

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prevention of Genital Herpes Simplex Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus - Senior Investigator - LCI, NIAID
R. McKenzie - Medical Officer - LCI, NIAID
B. Savarese - Research Nurse - LCI, NIAID
R. Kost - Medical Staff Fellow - LCI, NIAID
T. Heineman - Medical Staff Fellow - LCI, NIAID
P. Krause - Medical Staff Fellow - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI, NIAID
J. Lekstrom - Medical Staff Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*

R.L. Burke, M. Tigges, A. Langenberg, C. Dekker, (Biocine, Inc., Emmeryville, CA)
L. Corey, (University of Washington, Seattle)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Having completed over a decade of work on antiviral treatment of genital herpes, we turned to studies of disease prevention and immunotherapy. We completed an open 2 year phase 1 assessment of a new recombinant HSV-2 glycoprotein D vaccine in alum in 24 adults, some with and without prior HSV-1 and/or 2 infection. Vaccinations of 30 ug or 100 ug were given at 0, 1, 2, and 12 months and were associated with minimal local or systemic reactions. The vaccine induced excellent primary antibody in cellular immune responses and augmented preexisting humoral and cellular responses significantly. We studied antibody and T cells from previously uninfected subjects and showed that the gD2 epitopes that the antibody and cell recognize correspond to those in genital herpes patients. On the basis of these excellent responses we enrolled patients with recurrent genital herpes into a placebo-controlled vaccine trial in collaboration with the University of Washington. The goal was to determine whether boosted immunity leads to less frequent recurrences. Upon completing the study we documented that of 98 subjects, those who received the gD2 vaccine in alum experienced 1/4 - 1/3 fewer recurrences than those receiving alum alone. This is the first ever trial to demonstrate efficacy in the immunotherapy of a chronic viral infection. Rates of disease improvement, though, were lower than would be expected with acyclovir suppressive therapy so we began a dose seeking study and an efficacy study using gD2 combined with gB2 in a lipid adjuvant MF59. These studies will extend into 1995.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO AI 00589-04 LCI

PERIOD COVERED
October 1, 1992- September 30,1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Identification of Complement Resistance Determinants in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo, M.D., C.M., Senior Staff Fellow, NIAID, LCI
Suzanne Wenderoth, B.S., Biologist, NIAID, LCI

COOPERATING UNITS *(if any)*
Alan Cross, M.D., Walter Reed Army Institute of Research, Washington, D.C.

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	0.5	0.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Complement resistance is an important virulence determinant in the pathogenesis of bacterial gram-negative infections. A more complete understanding of complement resistance determinants may lead to novel therapies in the treatment of disease caused by these organisms. We have used transposon mutagenesis (TnphoA) to randomly mutate a clinical blood isolate of Escherichia coli (CP9,O4/K54/H5) that we are using as a model pathogen for extra-intestinal disease.This strain possesses a group 2 capsule and is highly serum resistant, as is characteristic of clinical isolates. Isogenic mutants have been identified that have an increased sensitivity to serum. Some of these mutants have been identified as being capsule-minus. We have defined at least 3 physical loci that are involved in capsule production in our strain and they are linked. DNA sequence analysis has demonstrated that we have disrupted at least 5 separate genes and that one has homology with capsular transport genes of Escherichia coli serotype K1, Haemophilus influenza and Neisseria meningitidis.

In vitro biologic studies have established that the K54 capsule protects against complement killing that is mediated via the alternative complement pathway. C3 binding is equal in capsule-positive and capsule-minus strains suggesting the mechanism of complement resistance conferred by the K54 capsule is different than that of the K1 capsule. Additional in vitro studies have demonstrated that the K54 capsule protects against killing by neutrophils. Three different animal models were used to test whether the K54 capsule conferred protection in vivo as well. In summary, these studies demonstrated that the possession of the K54 capsule resulted in a significant decrease of the LD50 in a mouse model of systemic infection, significantly decreased intra-vascular clearance and protected against killing in a local infection model. These studies also demonstrated that the K54 capsular polysaccharide may be one of the bacterial antigens responsible for inciting the cascade of events that leads to septic shock. They also demonstrated that other bacterial factors are induced in vivo that enhance survival and are important in the pathogenic process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00590-04 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Primary Immunodeficiency Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Warren Strober, M.D.,	Chief, Mucosal Immunity Section/LCI/NIAID
Others:	Eli M. Eisenstein, M.D.,	Clinical Associate, Mucosal Immunity Section/LIC/NIAID
	Kevin Chua, B.S.	Biologist, Mucosal Immunity Section/LCI/NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	1.25	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to define the immunopathogenetic mechanisms responsible for human immunodeficiency syndromes. During the past year we have focused on the functional capacities of T and B cells from patients with common variable immunodeficiency (CVI). In studies of CVI patient T cells, we showed that while purified CD4 T cells proliferate normally in response to proliferation by PHA, staphylococcal enterotoxin B (SEB) and anti-CD2 antibodies, these stimuli induce significantly less IL-2 than normal CD4+ T cells. On the other hand, immobilized anti-CD3 antibody and this stimulus in combination with anti-CD28 antibody induced CVI T cells to produce normal amounts of IL-2. These studies thus demonstrate that the IL-2 production defect in CD4+ T cells is due to a specific signalling pathway abnormality. In a second series of studies, in this case focused on B cell function in CVI patients, we showed first that circulating B cells express normal amounts of sIgM+, but greatly reduced amounts of sIgG+ and sIgA+; this result implies that patients have an *in vivo* defect of isotype switch differentiation. In other studies, we showed that upon stimulation of purified patient B cells with anti-CD40 antibody plus IL-10, patient B cells expressed Cμ, Cγ and Cα mRNA; furthermore, stimulation of cells with anti-CD40 plus IL-10, followed by stimulation with activated T cells (which express the CD40 ligand), led to partial restoration of IgG and IgA synthesis. These studies thus indicate that CVI B cells can be partially restored to normal function *in vitro*, suggesting that the cells are not irreversibly blocked from isotype and terminal differentiation.

PHS 6040 (Rev. 5/92)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00607-03 LCI

PERIOD COVERED

July 3, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cytokine Gene Expression in Mast Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section, LCI/NIAID
Other: Chad Oh, M.D. Medical Staff Fellow, LCI/NIAID
Yoseph Mekori, M.D. Fogarty Visiting Scientist, LCI/NIAID
Jaroslaw Dastych, Ph.D. Fogarty Visiting Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

NCI/FCRDC (Oppenheim)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.75	2.00	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Northern blot analysis shows that TCA3 is expressed after activation with either PMA/ionophore or high affinity IgE cross-linking in C57 cells. mRNA of TCA3 is induced within 2 hours, peaks at 3 hours, and disappears at 6 hours. Half-life of mRNA determined by adding actinomycin D is approximately 2 hours. These results suggest the TCA3 is regulated transcriptionally. Nuclear run-on assays show that the rate of TCA3 mRNA transcription is increased after activation consistent with transcriptional regualation. Promotor analysis demonstrated that the TCA3 gene is regulated transcriptionally in mast cells, and that the minimal promotor sequences are contained within the 0.082 kb upstream region of the TCA3 gene with a putative enhancer NF-kB element between -0.324 kb and -0.136 kb and a putative inhibitory element between 1.324 kb and 2.0 kb upstream from transcription start site.

Mast cells have also been transfected with a series of 5′ deletion mutants of TNF-α cat constructs to study the regulation of TNF-α via Fc$_{\varepsilon}$RI crosslinking.

Mast cells chemotax to the chemokines RANTES and MCAF in a dose-dependent manner. Parallel studies demonstrate that this chemotaxis is not accompanied by mast cell histamine release. When mast cells are activated through Fc$_{\varepsilon}$RI, chemotaxis can also be demonstrated to MIP-1α, and PF-4.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER AI 00621-03 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Transformation by Epstein-Barr Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jeffery I. Cohen, Senior Staff Fellow, LCI, NIAID

COOPERATING UNITS *(if any)*

D. Mosier, Scripps Research Institute

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Epstein-Barr virus (EBV) nuclear protein 2 (EBNA-2) is essential for B cell transformation by the virus. The goal of this project is to determine the mechanism of B cell transformation by EBV.

Injection of EBV-transformed human lymphocytes into SCID mice results in the appearance of fatal human B cell tumors containing EBV. Cell lines were developed that contained EBV mutants that were identical, except for different deletions in their EBV EBNA-2 gene. EBV mutants with deletions in the amino half of EBNA-2 had reduced transforming activity in vitro and produced tumors in mice more slowly, while a carboxy-terminal mutant had a transforming activity greater than or equal to wild-type and produced tumors more rapidly than wild-type. Therefore, EBNA-2 is important both for lymphocyte transformation in cell culture and B cell tumor growth in SCID mice.

EBV EBNA-2 contains a domain that directly activates transcription; a 14 amino acid region possesses much of the transcriptional activity and is essential for transformation by EBV. A chimeric virus was constructed that contained a portion of the transcriptional activation domain from the herpes simplex virus VP16 inserted in place of the 14 amino acid region in EBNA-2. This chimeric virus was able to transform B lymphocytes and transactivate expression of EBV and B cell genes. Mutation of a single amino acid in the 14 amino acid domain of EBNA-2 abolished transcriptional activation and B cell transformation. Thus, transcriptional activation is required for EBV-induced B cell transformation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00617-03 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Capsule Synthesis in a Pathogenic Escherichia coli Isolate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo M.D., C.M., Senior Staff Fellow, NIAID, LCI
Gagan Singh, Biologist, NIAID, LCI

COOPERATING UNITS *(if any)*

Susan Gottesman, Ph. D., Senior Investigator, LMP, NCI, NIH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The capsular antigens of Escherichia coli can be divided into group 1 and group 2 serotypes. It was previously thought that a single strain could possess only a single serotype.The biologic role of group 1 capsules has yet to be established. The majority of isolates of Escherichia coli responsible for extra-intestinal infections possess group 2 capsules. The majority are non-K1, but only the K1 antigen is widely held to be an important virulence factor. We are studying a non-K1 blood isolate (CP9,O4/K54/H5) as a model pathogen and have established that its K54 capsular polysaccharide is an important virulence factor in vitro and in vivo. The genes responsible for synthesizing several group 2 capsular polysaccharides have been cloned and their functional organization established. However, neither genes nor proteins involved in the regulation of group 2 capsule synthesis have yet been identified. In contrast, the regulation of the group 1 capsules produced by Escherichia coli K-12 strains, the K30 serotype and other members of the Enterobacteriaceae genera have been extensively studied. RcsA, RcsB and RcsF have been identified as positive regulators and RcsC and Lon as negative regulators. Therefore, we began an evaluation of the regulation of the group 2, K54 capsular polysaccharide. As a starting point, we assessed the role of the group 1 capsule regulators RcsA, RcsB and Lon on the regulation of group 2 capsule production.

Our studies have established that CP9 is capable of producing a group 1 capsule. RcsA, RcsB and Lon are present in this K54 background and regulate group 1 capsule expression in a fashion similar to that described for K-12 strains. Two independent group 2 capsule gene protein fusions (TnphoA) were used to evaluate the effects of these regulators on group 2, K54 capsule production. The results established RcsA as a negative regulator of the group 2 , K54 capsular polysaccharide. Its action appears to be mediated through RcsB and is greatest at 28 degrees centigrade, but decreases at higher temperatures. Furthermore, the results also suggest the existence of another Lon sensitive, negative regulator of group 2, K54 capsule production, which is active at higher temperatures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO AI 00650-02 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Chronic Hepatitis B Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus - Senior Investigator - LCI,NIAID
R. McKenzie - Medical Officer - LCI, NIAID
B. Savarese - Research Nurse - LCI, NIAID
R. Kost - Medical Staff Fellow - LCI, NIAID
T. Heineman - Medical Staff Fellow - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI,

COOPERATING UNITS *(if any)*

J. Hoofnagle, A. DiBisceglie, M. Fried, (NIDDK), K. Smiles (Oclassen Pharmaceuticals, San Francisco, CA), L. Corey (University of Washington, Seattle), J. Stotka (Eli Lilly Co., Indianapolis, Ind.)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been exploring the potential antiviral efficacy of a class of fluoropyrimidine nucleoside analogues. Initially we explored the drug FIAC for inhibition of CMV infection in AIDS patients. Antiviral activity was not seen at tolerated doses and studies turned to this drug's metabolic derivative, FIAU. Studies showed intolerance at high doses but excellent tolerance and profound inhibitory activity at lower doses against hepatitis B virus (HBV). The initial studies involved HIV positive patients with co-existing chronic HBV infection. Profound and even permanent reductions in circulating HBV DNA and antigens were achieved with oral FIAU at daily doses of 0.1, -.5, or 1 mg/kg for 14 days. We then turned to a broader dose modification study in normal patients with chronic HBV infection. Studies compared antiviral efficacy at .05, 0.1, 0.25 and 0.5 mg/kg per day for 28 days. We noted profound inhibition of HBV infection even at the lowest doses and in the absence of any substanial side effects. By 9 months after treatment 9 of 24 patients had lost HBV DNA; 2 of which lost HBeAg. Based on these findings 24 HIV-negative chronic HBV patients are being enrolled in a randomized study comparing 0.1 and 0.25 mg/kg per day for 6 months to determine long term safety and efficacy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO AI00651-02 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Recurrent Urinary Tract Infections In Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo, M.D., C.M., Senior Staff Fellow, NIAID, LCI
Suzanne Wenderoth, B.S., Biologist, NIAID, LCI

COOPERATING UNITS *(if any)*

Walter Stamm, M.D., Seattle, WA.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The most common site for extra-intestinal Escherichia coli infection is the urinary tract. There are a number of risk groups for this site of infection, one of which is young women in their sexually active years. Infection in this group accounts for 6-7 million office visits annually. Escherichia coli isolates are responsible for greater than 80-90% of these episodes. About 250,000 of these infections progress to acute pyelonephritis. In approximately 20% of these women frequent recurrences of infection (3-6/annum) follow the first episode. The morbidity, time lost from work and cost of this infectious syndrome is considerable. An improved understanding of the pathogenesis of this disease would be of significant importance. It is believed that the vast majority of recurrent UTIs are episodes of reinfection with different strains of E. coli rather than persistence of a single strain. We are employing pulsed field gel electrophoresis to generate DNA "fingerprints" of bacterial strains isolated from women with recurrent UTIs. This will allow us to definitively establish how frequently a single versus different strains are responsible for this pattern of infection.

Our studies to date have examined isolates from 23 women with recurrent urinary tract infections.The majority of symptomatic episodes in a given women are due to the same isolate. To assess whether the responsible strain was persisting within the gastro-intestinal tract or within the urinary tract, faecal isolates from 6 women were examined. This evaluation is consistent with the hypothesis that persistence within the gastro-intestinal tract is the mechanism by which the same isolate is responsible for recurrent UTIs in a given woman. These findings have significant implications for the treatment of women with recurrent UTIs.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00654-02-LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics, Biochemistry and Therapy of Candida albicans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John Bennett, M.D., Head, Clinical Mycology Section, LCI, NIAID
Others: Antonia Geber, M.D., Medical Staff Fellow, CMS, LCI, NIAID
Peter Williamson, M.D., Ph.D, Medical Staff Fellow, CMS, LCI, NIAID
K.J. Kwon Chung, Ph.D., Senior Scientist, CMS, LCI, NIAID

COOPERATING UNITS *(if any)*

Pfizer Central Research, Sandwich, England (Dr. Hancock)
George Washington University Medical Center (Dr. Tuazon)
University of Texas, Houston (Dr. Rex)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	1.5	2.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DNA upstream of the *Candida albicans* maltase gene has been sequenced and a potential promoter region inserted into an expression vector using the firefly luciferase gene as reporter. When the construct was used to transform *C. albicans*, ten-fold more luciferase activity was found under conditions which induce maltase than in the noninduced state. The method of intracellular processing which results in two forms of the maltase has been further elucidated. The enzyme is found synthesized as a 66 kDa pro-enzyme which is cleaved at approximately Val 475 to produce an enzyme 3 kDa smaller but 10 times more active towards the natural substrates, sucrose and maltose.
A multicenter trial comparison of fluconazole and amphotericin B for the therapy of candidemia in the nonimmunosuppressed host has stopped enrollment when the goal of 190 evaluable patients was obtained. Data are now being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00655-02-LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on pathogenic fungi

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John Bennett, M.D., Head, Clinical Mycology Section, LCI, NIAID
Others: Peter Williamson, M.D., Ph.D., Medical Staff Fellow, LCI, NIAID
K.J. Kwon-Chung, Ph.D., Senior Investigator, LCI, NIAID

COOPERATING UNITS *(if any)*

none

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Md.

TOTAL STAFF YEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The diphenol oxidase gene (DPO1) from Cryptococcus neoformans has been cloned on a 2.4 kb fragment and the sequence determined from been both cDNA and genomic DNA. The genomic sequence contains 14 introns, the longest of which contains 450 bases. DPO1 codes for 75 amino acids which have a sequence identity with the copper binding sites of various laccases, ascorbate oxidase and ceruloplasm. The purified enzyme is a 66 kDa glycoprotein which has the ability to oxidize dopamine to dopachrome (identified by HPLC-mass spectroscopy), the first intermediate during melanin synthesis. The protein appears to have several novel features for enzymes of its class including an unusual absorbance at 420 nm.

Three surface proteins from Cryptococcus neoformans have been isolated, one of which may be a secreted serine protease. These proteins are of interest as potential vaccine targets and for their role in capsular polysaccharide biosynthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00656-02-LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cryptococcal vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John Bennett, M.D., Head, Clinical Mycology Section, LCI, NIAID
Others: Peter Williamson, M.D., Ph.D., Medical Staff Fellow, CMS, LCI, NIAID

COOPERATING UNITS *(if any)*

John B. Robbins, M.D., Rachel Schneerson, M.D., LCMI, NICHD, Mary Smolskis, R.N., CC, NIH, Michael Polis, M.D., LIR, NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.6	PROFESSIONAL: 1.0	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Intramuscular injection of vaccine containing 50 mcg of type A cryptococcal capsular polysaccharide (glucuronoxylomannan, GXM) covalently linked to tetanus toxoid was well tolerated in more than 70 volunteers known to be seropositive for HIV-1. CD4 counts are being measured so that 30 volunteers will be in each of the following groups: CD4 <200, 200-500 and >500/cu mm. A single booster dose of 50 mcg will be given at 6 months and antibody titers followed one year. Followup of 50 vaccinated normal volunteers found that the 25 receiving the 50 mcg dose had a better preservation of IgG anti-GXM titer at 6 month follow up, the titer having fallen from 76 units at two weeks to 31 units, compared to the 25 volunteers receiving 25 mcg, who had a fall from 48 to 15 units/ml. Forty nine of the 50 normal volunteers have been bled at one year following initial immunization to determine peristence of antibody.

IgG purified from a vaccinated normal volunteer was injected into mice at a dose which induced anti-GXM levels comparable to those obtained in vaccinated normal volunteers. When injected intravenously with serotype A Cryptococcus neoformans, the mice were significantly protected against infection and death.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00657-02-LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic studies on Candida and Aspergillus species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: K.J.Kwon-Chung, Research Microbiologist, Clinical Mycology Section, LCI,NIAID
Others: Mark J. Parta, Medical Staff Fellow, Clinical Mycology Section, LCI, NIAID
Yun C. Chang, Staff Fellow, Clinical Mycology Section, LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Aspergillus fumigatus, a ubiquitous fungus, causes substantial morbidity and mortality in humans, principally those with compromised immunity by genetic predisposition or iatrogenic immunosuppression. Although primarily a respiratory tract pathogen, via inhalation of airborn spores, little is known about the precise events and interactions with the host that lead to successful survival and propagation of the fungus. We have cloned a gene controlling the hydrophobicity of *A. fumigatus* conidia in order to study its role in the survival and growth of the fungus in the host tissue. Polymerase chain reaction(PCR) method was used to clone a hydrophobin which has been identified in other nonpathogenic fungi.The sequence of a 400 bp DNA fragment, generated by PCR from *A. fumigatus* DNA, showed 80.6% similarity to a hydrophobin gene (*ROD A*) of *A.nidulans*. A lambda genomic DNA library was screened with the 400 bp fragment and the genomic subclone containing this 400 bp DNA was sequenced. The amino acid sequence showed 8 cysteines arranged in a pattern similar to that observed in the *ROD A* protein of the conidial wall in *A.nidulans* and to three hydrophobic cell wall proteins of a mushroom species, *Schizophillum commune*.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

AI 00674-01 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Varicella-Zoster Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jeffrey I. Cohen
Others: T. Heineman, Medical Staff Fellow, LCI, NIAID
K.E. Seide., Bio. Lab. Tech., LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.7	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Varicella-zoster virus (VZV) is the etiologic agent of chickenpox and herpes zoster. We have developed a system to generate recombinant VZV by transfecting tissue culture cells with four overlapping VZV cosmid DNAs.

Mutations have been engineered into the cosmids that result in VZV that is unable to express the viral thymidylate synthetase, glycoprotein V, or ribonucleotide reductase genes. Viruses that are unable to express the former two genes grow at similar rates as the parental virus, while virus that is unable to express the ribonucleotide reductase gene grows at a slower rate than wild-type virus. Selected VZV mutants will used to inoculate guinea pigs to determine if the viruses have lost the ability to establish latency in the central nervous system.

Foreign genes have been inserted into the VZV genome. The E. coli beta-galactosidase gene, has been inserted into the VZV genome, and the resultant virus produces plaques that stain blue with X gal. This virus has been inoculated into guinea pigs to determine the types of cells that are able to support viral replication and establishment of latency. The herpes simplex virus (HSV) glycoprotein D (gD) gene, encoding a major neutralizing antigen, has been inserted into VZV and the resulting virus expresses high levels of HSV gD on the surface of infected cells. Animals will be inoculated with VZV expressing HSV gD to determine if they are protected against infection with HSV.

A cell line has been developed that secretes VZV glycoprotein II (gpII), an important neutralizing antigen. Guinea pigs will be injected with recombinant VZV gpII and challenged with live VZV to determine if they are protected from infection with VZV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
AI 00680-01 LCI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Novel Cytokines From Activated Macrophages

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Joshua M. Farber, NIAID Investigator, LCI/NIAID
Other: Fang Liao, Visiting Fellow

COOPERATING UNITS *(if any)*
Padmavathy Vanguri, University of Maryland; Stephen Wesselingh, Johns Hopkins University; Keith Peden, LMM, NIAID

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Cytokine Biology Unity

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.83	0.83	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall goal of the project is to identify and characterize macrophage products of potential importance in immune and inflammatory responses in order to manipulate these responses for clinical benefit.

Differential screening of a cDNA library prepared from a mouse macrophage-like cell line led to the identification of 11 mRNA species of increased abundance following exposure to gamma-interferon. Two of these mRNA species, designated Mig and Crg-2 encode previously undescribed members of a newly-defined family of small secreted proteins, termed chemokines, that includes platelet factor 4, melanoma growth stimulatory activity (MGSA/gro) and IL-8 among others. Using the mouse MuMig cDNA probe, a new human member of the family has been discovered.

Work to date has demonstrated that the MuMig and HuMig mRNAs accumulate in monocytic cells specifically in response to gamma interferon, while Crg-2/IP-10 can respond to alpha interferon and to lipopolysaccharide as well. Following intraperitoneal injection of gamma interferon into mice, MuMig and Crg-2 are induced in a range of tissues including most prominently liver, lung, spleen and heart.

Antibodies have been raised in rabbits against MuMig, HuMig and Crg-2 using proteins expressed in and purified from bacteria as antigens, Chinese hamster ovary cell lines were derived that overexpress the MuMig, HuMig, Crg-2 and IP-10 proteins. As predicted, each of the proteins is secreted. Using a Western blot assay, the MuMig and HuMig proteins have been purified to near-homogeneity. By PAGE, the Mig proteins show multiple species with mobilities corresponding to 16-20 kDa for MuMig and 12-35 kDa for HuMig.

Ongoing work is concentrating on a more detailed analysis of the cell types that produce the MuMig/HuMig and Crg-2/IP-10 proteins; on the biochemical characterization of the purified Mig proteins; on the identification and molecular cloning of the Mig receptor(s); on determining the biological activities of MuMig/HuMig and Crg-2/IP-10; and on identifying additional new members of the chemokine family.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00687-01 LCI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Function Analysis of the Immediate-Early Protein IE62 of Varicella-Zoster

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.P. Perera, Senior Staff Fellow, LCI, NIAID
Other: S.E. Straus, Senior Investigator, LCI, NIAID

COOPERATING UNITS *(if any)*

J.D. Mosca, Henry M. Jackson Foundation, Rockville, MD, G.S. Hayward, Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Varicella zoster virus (VZV), a neurotropic alphaherpesvirus, is the etiologic agent of two distinct clinical syndromes: chickenpox (varicella) and shingles (zoster). VZV is an enveloped virus with a linear double-stranded genome of approximately 125,000 base pairs (bp). The complete genomic nucleotide sequence of VZV has been determined and predicts approximately 70 unique genes. The expression of VZV genes appear to be temporally regulated and three putative kinetic classes have been defined for VZV genes; namely immediate early, early and late. Evolving evidence suggest that the putative immediate early gene product of ORF62 (IE62) to be the dominant regulatory protein in VZV. In addition, the ORF62 gene has been shown to be transcriptionally active during the latent phase of VZV infection in ganglia, thus suggesting a potential role for this gene in the maintenance of latency.

Current efforts focus on the structure-function analysis of IE62 regulatory protein using a variety of genetic approaches. In transient expression assays, the IE62 protein has been shown to be a potent promiscuous transactivator of both VZV genes and heterologous genes. The activation domain of the 1310-amino acid IE62 protein has been localized to a 75-amino acid region in the N-terminus of the molecule. Using saturation and site specific mutagenesis further dissection of the 75-amino acid activation domain has been undertaken to identify functionally critical residues within the activation domain. Furthermore, our recent studies have illustrated that the IE62 -dependent activation of responsive promoters are mediated by a unique mechanism involving the TATA element of the promoter. Studies are in progress to delineate the interactions of IE62 with the transcriptional machinery of the Pol II promoters. In addition, the promoter functions of IE62 gene are being investigated. Preliminary findings indicate enhanced promoter activity in neuronal cells and a 80-bp region in the promoter has been identified as being capable of confering neuronal specificity to the ORF62 promoter. Isolation and identification of neuronal specific transcription factors that interact with the 80-bp promoter element are currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI-00688-01

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Herpesvirus interaction with cell surface molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard K. Williams, Ph.D., Senior Staff Fellow, LCI/NIAID
Others: Stephen E. Straus, M.D., Senior Investigator, LCI/NIAID

COOPERATING UNITS *(if any)*

none

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms of binding and entry of varicella zoster virus (VZV) into cells are unknown. We are investigating the binding of VZV to cell membrane preparations from a variety of cultured cell-lines, to isolated proteins, and to synthetic peptides. Inhibition of VZV infectivity in diploid human lung cells is being used to assess potential receptor-mimicking molecules for ability to block VZV binding and infection of cells. Individual VZV glycoproteins produced in a mammalian cell expression system are used to examine binding to potential components of the VZV receptor system. Monoclonal and polyclonal antibodies to cell membrane proteins are being developed to identify the specific cell surface molecules that serve as receptors for VZV. The host range of the VZV receptor is being examined prior to transfer of potential receptor genes in order to confer VZV susceptibility in cell lines previously resistant to VZV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00045-23 LCI

PERIOD COVERED

October 1992 - September 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Interaction of Antibody and Complement on Production of Immune Damage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Michael Frank, M.D.	Chief	LCI, NIAID
	Carl Hammer, Ph.D.	Senior Investigator	LCI, NIAID
	Tom Waytes, M.D.	Medical Staff Fellow	
	Robin McKenzie, M.D.	Medical Staff Fellow	

COOPERATING UNITS *(if any)*

Bernard Moss, LVD, NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Terminated 1991 - 1992

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00469-07 LCI

PERIOD COVERED
October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Comlement: Studies in Viral Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I. Robin McKenzie, M.D. Medical Staff Fellow LCI, NIAID

COOPERATING UNITS *(if any)*
Brian Murphy, M.D., LID, NIAID,
Bernard Moss, M.D., LVD, NIAID
Michael Frank, M.D., Duke University

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.3	0.3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Terminated 1992 - 1993

LABORATORY OF CELLULAR AND MOLECULAR IMMUNOLOGY

ANNUAL REPORT FY 1993

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PHS-NIH
Summary Statement
Office of Chief
Laboratory of Cellular and Molecular Immunology
October 1, 1992 through September 30, 1993

Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. Its major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical basic cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up in vitro analogs in tissue culture, and determine the molecular basis for the phenomena. The laboratory employs techniques from the disciplines of cellular immunology, molecular biology, and protein biochemistry.

During the 7th year, the laboratory has pursued eight major areas of research: the molecular nature of costimulation, the ability of B cells to present antigen to naive T cells, stochastic vs instructional models of T cell development, the expression of IL-2 receptor γ chain in developing T cells, the role of terminal nucleotidyl transferase in thymic development, the mechanisms and consequences of self tolerance, the mechanisms maintaining T cell memory, and the nature of the thymic stem cell.

T Cell Activation

Studies on the activation of normal murine T cell clones of the Th1 type have shown that two signals are required to stimulate the cells to make interleukin-2 (IL-2) and divide. One signal is given through the antigen-specific receptor, which is uniquely expressed on each clone. The other signal is called costimulation and is delivered through the CD28 receptor, which is expressed in the same form on all mouse T cells. Our major objective for the past two years has been to discover the molecular basis for costimulation. Using an antibody against CD28 to mimic costimulation and an antibody against the β chain of the T cell receptor to mimic antigenic stimulation, we have studied the way in which costimulation synergizes with T cell receptor occupancy to enhance IL-2 production. In the absence of costimulation 2×10^4 T cells secrete about 0.2 units of IL-2. In the presence of anti-CD28 antibody the amount increases one hundred-fold. T cell clones were stably transfected with reporter constructs containing the murine IL-2 enhancer/promoter region joined to a luciferase gene in order to examine the effects of costimulation on DNA transcription. Although antibody to the T cell antigen-receptor increased luciferase production substantially in such transfectants, addition of anti-CD28 antibody gave no further increase, suggesting that costimulation does not augment IL-2 production by increasing the rate of transcription. The stability of IL-2 messenger RNA was then examined by stimulating the cells for 4 hours and stopping transcription with cyclosporin A. Under these conditions mRNA generated with anti-T cell receptor antibody stimulation was found to be relatively unstable with a half-life of 30 minutes. Addition of anti-CD28 significantly stabilized the mRNA, preventing degradation for at least one hour, followed by decay with a half-life of 30-40 minutes. Integration of the mRNA decay curves in one experiment suggested that the different stabilities could fully account for the difference in IL-2 accumulation with and without anti-CD28 over that same time period. Thus, the major effect of costimulation through the CD28 receptor appears to be to increase the stability of the IL-2 message.

(S. Umlauf, S. Kitagawa, M. Manoussakis, and R.H. Schwartz, LCMI, NIAID)

T Cell Subsets

The initiation of an adaptive immune response involves the presentation of antigen to T lymphocytes. Which cells are capable of carrying out this process is still not completely decided. Consensus exists for the role of interdigitating dendritic cells, but the role of the B lymphocyte is more controversial. During the past year we addressed this issue by using T cell receptor transgenic mice as a source of antigen-specific, naive $CD4^+$ T cells and testing the ability of these cells to be stimulated to produce interleukin-2 (IL-2) by resting and activated B cells. Activated B cells were capable of stimulating such T cells but resting B cells were not. When compared to dendritic cells, however, activated B cells were less efficient, requiring more cells per culture well and stimulating less IL-2 per T cell. This deficiency appeared to be caused by an inadequate amount of costimulatory activity, because it could be corrected by the addition of an anti-CD28 antibody but not by increasing the concentration of antigen. Resting B cells also functioned in the presence of anti-CD28 antibody suggesting that the ability to deliver costimulation is the major limitation that needs to be overcome in order for B cells to stimulate naive $CD4^+$ T cells.

(D. Cassell, A. Bendelac, L. Chiodetti, and R.H. Schwartz, LCMI, NIAID)

T Cell Differentiation

Studies with T cell receptor (TCR) transgenic mice have established a correlation between the specificity of the TCR for MHC class I or II molecules and the thymic development of the $CD8^+4^-$ and $CD4^+8^-$ mature T cell lineages, respectively. To explain this linkage two models have been proposed: an instructional model, in which MHC class I or II recognition during positive selection signals through the TCR complex for commitment to a particular lineage; and a second, stochastic model, in which random inactivation of CD4 or CD8 is followed by positive selection based on TCR and coreceptor affinity. These two models can be distinguished as the latter predicts the existence of committed intermediates which will not be selected i.e. $CD4^+8^-$ T cells with a TCR specific for MHC class I molecules and $CD8^+4^-$ T cells with a TCR specific for MHC class II molecules. During the past three years Dr. Fowlkes' laboratory has searched for these intermediates in conjunction with Dr. Ellen Robey by crossing transgenic mice constitutively expressing the CD8 molecule under the control of a CD2 promoter to T cell receptor transgenic mice or mice devoid of MHC class II molecules as a result of DNA-mediated gene targeting. In the former case no evidence was found for the rescue of $CD4^+$ intermediates with the addition of constitutive CD8 to a TCR specific for an MHC class I molecule; however, the CD8 molecule did rescue a small percentage of $CD4^+$ T cells in the class II "knock out" mice suggesting that at least some T cells can commit to the CD4 lineage in the absence of instruction by MHC class II molecules. Another observation made recently, which possibly contributes to the argument over stochastic versus instructional models, is the finding that selection of a $CD8^+4^-$ T cell subset can occur in a transgenic mouse expressing a TCR specific for a cytochrome c peptide plus an MHC class II molecule. This receptor is normally selected to become a $CD4^+$ T cell when the A^b MHC class II molecule is expressed in the thymus. However, if the E^b class II molecule and the K^b MHC class I molecule are also expressed, then $CD8^+$ T cells emerge as well. This event is not a consequence of an E molecule-derived peptide binding to the K^b class I molecule to generate an alternative positively selecting ligand, because antibodies to the intact E molecule blocked the generation of the $CD8^+$ T cells. The current working hypothesis is based on the observation that the level of TCR expression was found to be higher on $CD4^+8^+$ T cells in the presence of E^b. It is speculated that stochastically generated $CD8^+$ T cells are selected on the K^b class I molecule when the TCR expression is high, but not when it is low. Experiments to test this model are currently underway. Finally, in two new initiatives, Dr. Fowlkes' lab is collaborating with Dr. W. Leonard's lab to study the expression of the IL-2 receptor gamma chain in thymic development, and Dr. F. Alt's lab to study the effect of the absence of terminal deoxynucleotidyl transferase on positive and negative selection.

(E. Schweighoffer and B.J. Fowlkes; LCMI, NIAID)

T Cell Tolerance and Memory

The laboratory of Dr. Matzinger has continued to study the mechanisms of self tolerance and memory and the nature of the thymic stem cell. In collaboration with Dr. David Gray, it was previously demonstrated that the memory cytotoxic T cell response to the male-specific antigen H-Y rapidly declined if the primed T cells were transferred to an irradiated naive recipient. This result was interpreted as a loss of T cell memory in the absence of antigen. Persistence of antigen was postulated to be through maintenance of antigen-antibody complexes on the surface of follicular dendritic cells. If this were true, then the persistence of T cell memory should require the presence of B cells. To test this idea mice deficient in B cells were created by reconstituting SCID mice with fetal thymus grafts. The grafts repopulate the T cell compartment but do not generate a source of B cells. These "B-less" mice were competent to generate both T cell proliferative and IL-2 responses to soluble protein antigens, but when tested for long term memory they failed to show it. The frequency of antigen-specific IL-3 producing cells dropped steadily from two weeks after immunization while SCIDS reconstituted with B cells in addition to the fetal thymus grafts maintained their ability to mount a memory response for months. Thus, T cell memory requires the presence of B cells.

In the areas of tolerance and thymic stem cells some preliminary observations have been made which are very interesting and are being actively pursued. A fraction of neonatal female mice injected with male dendritic cells were primed instead of tolerized, in contrast to what normally happens when whole spleen cells are injected, i.e., tolerance induction up to 12 days of age. This suggests that the small number of mature T cells present at birth are intrinscially activatable if presented with antigen on the right presenting cells. In the area of maternal tolerance, fetal cells were detected late in gestation in the mothers' spleen, lymph nodes and occasionally thymus, using a quantitative PCR technique to detect the Y chromosome. These cells disappeared around the time of birth. Their effect on the maternal immune system is currently being assessed. Preliminary experiments suggest that they do not prime the mothers to the H-Y antigen. Finally, in the study of the thymic stem cell a new assay system has been developed to examine the timing of stem cell commitment by isolating day 11 fetal thymocytes and adding them to thymic organ cultures. Preliminary observations suggest that the day 11 cells can be divided into two distinct populations, one that proliferates rapidly and one that does not.

(L. Bonney, L. Yuan I, O. Lantz, and P. Matzinger; LCMI, NIAID).

Administrative, Organizational, and Other Changes

During the past year Dr. Elizabeth Bonney, Dr. Oliver Lantz, Dr. Lin Yuan I, Dr. Edina Schweighoffer and Ms. Leah Evans joined the laboratory and Dr. Delanie Cassell and Ms. Mary Cyr left the laboratory.

Honors, Awards and Scientific Recognition

Dr. Schwartz is a member of the editorial boards of Science International Immunology, Immunology Today and the International Journal of Cell Cloning.

During the past year he was an invited speaker at the MD Anderson Symposium on the Immunobiology of Cancer : Cellular and Molecular Mechanisms, held in Houston Texas; an invited speaker in the Health Sciences Center Seminar Series at the University of Virginia in Charlottesville, VA; a lecturer in the FDA Staff College course on Immunosuppressive Drug Development and Transplantation Science held in Rockville, MD; a guest lecturer in the Inserum Advanced Immunology course held at the Hopital Necker in Paris, France, a seminar speaker in the Department of Microbiology at Columbia Medical School in NYC, NY; Co-Chairman of a symposium at a meeting on Cytokines and Cytokine Receptors in Health and Disease sponsored by the NHLBI, NIH; a seminar speaker in the Honors Program lecture series at NYU Medical School, NYC, NY; a seminar speaker in the Department of Pathology at the University of Pennsylvania in Philadelphia, PA; an invited speaker at the U.S.-Japan Cooperative Research Program meeting on Self-Non-self Discrimination in Transplantation and Autoimmunity sponsored by the NCI, NIH, in Kona Hawaii; a symposium speaker at the meeting on Specific Immunotherapy of Cancer with Vaccines held in Washington, DC.; a seminar speaker and guest lecturer in the graduate program at Johns-Hopkins Medical School, Baltimore, MD; a seminar speaker in the Department of Surgery at the Massachusetts General Hospital, Boston, MA; a symposium chairman at the Nobel Conference an Autoimmunity: on old enigma in a new light, held in Stockholm, Sweden; and a symposium chairman at a meeting entitled Towards Selective Immunotherapy of Autoimmune Diseases held in Milano, Italy.

Dr. B.J. Fowlkes serves on the editorial board of the *Journal of Experimental Medicine* and is a frequent reviewer for *Nature*, *Science*, *The Journal of Immunology*, and *International Immunology*. She serves on the Committee on the Status of Women for the American Association of Immunology, the NIH Director's Task Force for Recommendations to Improve the Status of NIH Intramural Women Scientists, and as the Women Scientist Advisor to the Scientific Director of NIAID. During the past year, Dr. Fowlkes was invited to chair the *Minisymposium on T Cell Unresponsiveness* at the AAI/CIS joint meeting in Denver, Colorado, and a workshop on *Lymphocyte Differentiation and Lineage Commitment* in the NIH Research Festival. In addition, she was an invited speaker at the FASEB Summer Research Conference in Saxton's River, Vermont, on *Lymphocytes and Antibodies*; at the Fourth Annual Symposium on Transplantation held at Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois; and at the WHO/HHS/IASIA meeting on *Establishing a Research Agenda for Immunology and Aging* at NIH.

Dr. Fowlkes was invited to give research seminars on T cell development, selection and/or tolerance to HHMI-NIH Research Scholars at NIH Howard Hughes Medical Institute (Bethesda, MD); Department of Microbiology, Mount Sinai-Medical Center (New York, NY); Department of Medicine, North Shore University Hospital (Manhasset, NY); Immunology Affinity Group, Scripps Research Institute (La Jolla, CA); Department of Biology, University of California (San Diego, CA); Department of Microbiology, Uniformed Services University of the Health Sciences, Laboratory of Viral and Molecular Pathogenesis, NINDS, NIH; Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati Medical Center (Cincinnati, OH); Immunology Program, Boston University School of Medicine (Boston, MA); Division of Immunology, Beth Israel Hospital, Harvard Medical School (Boston, MA); and the Committee on Immunology, University of Chicago (Chicago, IL). She served on the faculty for courses in *Biochemistry and Molecular Biology of Immune Responses* in the Department of Biochemistry and Molecular Biology at George Washington University (Washington, DC) and *Fundamental Immunology and it Application to the Study of Schistosomiasis* held by the International Union of Immunological Societies and the Schistosomiasis Research Project (Sharm El Sheikh, Red Sea, Egypt)

Dr. Matzinger is a member of the editorial boards of Immunobiology, Seminars immunology, International Archives of Allergy and Immunology and Current Opinion in Immunology. She also is a regular contributor to "Real Science" a monthly letter for high school students.

During the past year she was an invited chairperson and speaker at the British Society for Immunology symposium on Autoimmunity, London, England; the Denver Immunology Research Symposium, Denver, CO; the International Workshop on B and T cell Memory, Bethesda, MD. and a conference on "Towards Selective Immunotherapy of Autoimmune Diseases, Milano, Italy. She was a co-organizer and participant in the Workshop on Immune Memory: Experiments, Interpretations and Models at the Santa Fe Institute, New Mexico.; she was founding teacher at the First and Second Central European Summer School of Immunology in Piestany, Slovakia and Budejovice, Ceske. She was lecturer in the american Association of Immunology's course in Advanced Immunology, Colorado Springs, CO and the keynote speaker for the Summer Western Conference, Berkely, CA. She was invited to present research seminars at the University of Pennsylvania, School of Medicine, Philadelphia, PA; the Wistar Institute, Philadelphia, PA; The University of Massachusetts, Worcester, MA; the Santa Fe Institute, Santa Fe, NM; the Pittsburgh Cancer Institute, Pittsburgh, PA.; the Department of Molecular and Cell Biology, University of California at Berkely, CA.: the School of Medicine, University of California at San Francisco, CA; Genentech, South San Francisco, CA.; Genetics Institute, Boston, MA.; Tufts University, Boston, MA.; and the Department of Biology, University of California at San Diego, La Jolla, CA. She is a consultant for the Genetics Institute, Boston, MA and Marion Merrill Dow, Kansas City, MO.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00485-07 LCMI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

T Cell Activation

PRINCIPAL INVESTIGATOR Dr. Ronald Schwartz, Chief LCMI, NIAID

Others:

Scott Umlauf	Staff Fellow	LCMI
Satoru Kitagawa	Visiting Fellow	LCMI
Menelaus Manoussakis	Visiting Fellow	LCMI

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Activation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	3.5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Studies on the activation of normal murine T cell clones of the Th 1 type have shown that two signals are required to stimulate the cells to make interleukin-2 (IL-2) and divide. One signal is given through the antigen-specific receptor, which is uniquely expressed on each clone. The other signal is called costimulation and is delivered through the CD28 receptor, which is expressed in the same form on all mouse T cells. Our major objective for the past two years has been to discover the molecular basis for costimulation. Using an antibody against CD28 to mimic costimulation and an antibody against the chain of the T cell receptor to mimic antigenic stimulation, we have studied the way in which costimulation synergizes with T cell receptor occupancy to enhance IL-2 production. In the absence of costimulation $2x10^4$ T cells secrete about 0.2 units of IL-2. In the presence of anti-CD28 antibody the amount increases one hundred-fold. T cells clones were stably transfected with reporter constructs containing the murine IL-2 enhancer/promoter region joined to a luciferase gene in order to examine the effects of costimulation on DNA transcription. Although antibody to the T cell antigen-receptor increased luciferase production substantially in such transfectants, addition of anti-CD28 antibody gave no further increase, suggesting that costimulation does not augment IL-2 production by increasing the rate of transcription. The stability of IL-2 messenger RNA was then examined by stimulating the cells for 4 hours and stopping transcription with cyclosporin A. Actinomycin D could not be used as it was found to artificially stabilize IL-2 message on its own. Under these conditions mRNA generated with anti-T cell receptor antibody stimulation was found to be relatively unstable with a half-life of 30 minutes. Addition of anti-CD28 significantly stabilized the mRNA, preventing degradation for at least one hour, followed by decay with a half-life of 30-40 minutes. Integration of the mRNA decay curves in one experiment suggested that the different stabilities could fully account for the difference in IL-2 accumulation with and without anti-CD28 over that same time period. Thus, the major effect of costimulation through the CD28 receptor appears to be to increase the stability of the IL-2 message.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00486-07 LCMI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
T Cell Differentiation

PRINCIPAL INVESTIGATOR	B. J. Fowlkes	Senior Investigator and Section Chief	LCMI, NIAID
Others:	E. Schweighoffer	Visiting Fellow	LCMI, NIAID
	E. O'Connell	Technician	LCMI, NIAID
	H. Dinh	Stay-in-school	LCMI, NIAID

COOPERATING UNITS *(if any)* Flow Cytometry Section, BRB, NIAID (R. Swofford); Animal Care Branch, DIR, NIAID (P. Golway); Department of Molecular and Cell Biology, University of California, Berkeley (E. Robey); Section on Pulmonary and Molecular Immunology, NHLBI (W. Leonard); Howard Hughes Medical Institute, Children's Hospital (F. Alt).

LAB/BRANCH
Laboratory of Cellular and Molecular Immunology

SECTION
Thymocyte Differentiation Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.25	2.0	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

During T cell development, thymocytes that are able to recognize self MHC are signaled to mature (positive selection), whereas those capable of self reactivity die (negative selection). Many of our studies deal with these selection processes that result from interactions that occur between T cell receptors (TCR) on developing T cells with MHC on antigen presenting cells in the thymic microenvironment. Experiments have been performed in order to determine how and when T cells commit to the CD4 and CD8 lineages. Since CD4 and CD8 T cells arise from a common $CD4^+8^+$ precursor, coreceptor (CD4 or 8) expression in mature T cells appears to be linked to MHC class recognition by TCR. Experiments involving TCR and CD8 transgenic mice were used to test whether lineage commitment occurs by a stochastic process or is directed by the class of MHC that the TCR and coreceptor coengage during the course of thymic selection. Our studies show that at least some T cells can be selected on more than one MHC and imply that positive selection is a multi-step process involving a stochastic event that randomly modulates one of the coreceptors on $CD4^+8^+$ immature T cells. In other studies, we find that at least a few T cells can be rescued with an inappropriate coreceptor (inappropriate for the MHC specificity of the TCR on the same T cell) when the appropriate coreceptor is expressed as a transgene. Most of the results suggest that there is a stochastic component regulating coreceptor expression in T cell development; however, the data do not as yet establish whether this is a major pathway for the bulk of T cells and/or whether the stochastic process actually mediates lineage commitment.

Collaborative studies with W. Leonard of NHLBI have established that IL-2Rγ is expressed in the thymus. Studies are under way to explain how mutation of this gene could account for the failure to generate mature T cells that is observed in human severe combined immunodeficiency disease (SCID). Collaborative studies are in progress with F. Alt using terminal deoxynucleotidyl transferase (*TdT*)-deficient mice in order to establish whether T cells that lack N regions in their TCR variable regions undergo normal thymic development and selection and/or mediate normal antigen recognition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00581-04 LCMI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

T Cell Tolerance and Memory

PRINCIPAL INVESTIGATOR Polly Matzinger, Special Expert

Others:

Elizabeth Bonney	Medical Staff Fellow	LCMI, NIAID
Lin Yuan I	Fogarty Visiting Fellow	LCMI, NIAID
Olivier Lantz	Fogarty Visiting Fellow	LCMI, NIAID
John P. Ridge	GS-11 Technician	LCMI, NIAID
David Gray	Reader	Hammersmith Hospital London

COOPERATING UNITS *(if any)*

LI

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Tolerance Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL WOMAN/MAN-YEARS:	PROFESSIONAL:	OTHER:
4/2.2	5.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

This year, the lab has continued to study the mechanisms and consequences of self tolerance, the mechanisms maintaining T cell memory, and the nature of the thymic stem cell.

TOLERANCE: *1) Neonatal tolerance:* Our discovery that virgin T cells are tolerized by B cells hints that neonatal mice may not be uniquely tolerizable. If the number of T cells is low; if they are all virgin; and if the injection contains many B cells, then most T cells will encounter antigen on a B cell and be tolerized, yet they should respond to professional APCs. We therefore immunized neonatal female mice with male dendritic cells. Some responded as early as day 2 after birth. Thus, neonatal T cells are simply virgin T cells tolerized by B cells and immunized by professional APCs. *2) Maternal tolerance:* We analyzed cell traffic across the placenta. Using quantitative PCR for the Y chromosome, we found male cells in the thymus, spleen, and lymph nodes of pregnant mice, but by limiting dilution analysis, we found that female mice are not immunized by their male progeny. We are now testing whether they are tolerized. *3) Results of tolerance in $T\gamma$ mice:* We are testing thymic "positive selection" by asking which haplotypes support the development of a $T\gamma$ TCR derived from an F_1 mouse. Positive selection models predict that only one of the parental haplotypes permits development whereas we predict that these T cells will develop in both.

THE THYMIC STEM CELL: *1) Is it precommitted?* Eleven-day-old fetuses (one day after stem cell colonization begins) contain about 2000 cells divided into two distinct populations: one of them rapidly proliferating. We measure the developmental potential of these cells using thymus and spleen organ cultures. By measuring the mature cell types produced by a stem cell, we hope eventually to determine the nature and timing of commitment signals. *2) How long does it last?* Some fetal thymuses transplanted into SCID mice are producing T cells months later. We cannot predict which particular thymuses will be producing at any time, and our current hypothesis is that the stem cell may cycle and that we sometimes catch the thymuses at the low ebb of the cycle and sometimes at its peak.

T CELL MEMORY: We have previously shown that T cell memory in both rats and mice requires the persistence of antigen. If Ag is deposited as Ag-Ab complexes on follicular dendritic cells in germinal centers, than T cell memory should require the presence of B cells. To test this, we created B cell-deficient mice by grafting SCID mice with fetal thymuses. Such B-less mice responded to soluble protein antigens but did not maintain long-term memory. Using limiting dilution assays, we find that the frequency of responding cells drops steadily from two weeks after immunization, whereas SCIDs containing B cells maintain their ability to mount a memory response for months. Thus, T memory requires the presence of B cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00613-03 LCMI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

T Cell Subsets

PRINCIPAL INVESTIGATOR Dr. Ronald Schwartz, Chief LCMI, NIAID

Others:

Albert Bendelac	Visiting Associate	LCMI, NIAID
Delanie Cassell	IRTA	LCMI, NIAID
Lynda Chiodetti	GS-12 Technician	LCMI, NIAID

COOPERATING UNITS *(if any)*

Flow Cytometry Section, Biological Resources Branch, NIAID
Animal Care Branch, NIAID

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Activation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The initiation of an adaptive immune response involves the presentation of antigen to T lymphocytes. Which cells are capable of carrying out this process is still not completely decided. Consensus exists for the role of interdigitating dendritic cells, but the role of the B lymphocyte is more controversial. During the past year we addressed this issue by using T cell receptor transgenic mice as a source of antigen-specific, naive $CD4^+$ T cells and testing the ability of these cells to be stimulated to produce interleukin-2 (IL-2) by resting and activated B cells. Activated B cells were capable of stimualting such T cells but resting B cells were not. When compared to dendritic cells, however, activated B cells were less efficient, requiring more cells per culture well and stimulating less IL-2 per T cell. This deficiency appeared to be caused by an inadequate amount of costimulatory activity, because it could be corrected by the addition of an anti-CD28 antibody but not by increasing the concentration of antigen. Resting B cells also functioned in the presence of anti-CD28 antibody suggesting that the ability to deliver costimulation is the major limitation that needs to be overcome in B cell activation of naive $CD4^+$ T cells.

LABORATORY OF HOST DEFENSES
1993 Annual Report
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PHS-NIH

Summary Statement

LABORATORY OF HOST DEFENSES

National Institute of Allergy and Infectious Diseases

October 1, 1992 to September 30, 1993

Introduction.

The focus of work in the Laboratory of Host Defenses, (LHD) is (1) to study the basic mechanisms of protection against bacterial and fungal infection; (2) to study the physiology, biochemistry and molecular biology of phagocytic cells (blood neutrophils, monocytes, eosinophils, and fixed tissue macrophages) in host defense against bacteria and fungi; (3) to determine the role and effect of cytokines on both phagocytic cells and upon the general process of inflammation; (4) to investigate the cellular, biochemical and genetic basis of diseases leading to recurrent bacterial and fungal infections, particularly diseases affecting phagocytic cells function (chronic granulomatous diseases, hyper-IgE-recurrent infection [Job's] syndrome, specific granule deficiency, leukocyte adhesion deficiency, cyclic neutropenia and others); (5) to develop new animal models of human diseases which lead to recurrent infections; and (6) to develop new treatments for host defense defects leading to recurrent infections including new antibiotic or cytokine therapy or prophylaxis against infection, new diagnostic approaches for infections, and the development of gene therapy for such inherited immune deficiencies.

During the past year the Laboratory has continued and expanded upon clinical studies in patient cohorts collected at NIH over more than 20 years. Studies have been performed in patients and normal volunteers regarding the physiology and biochemistry of phagocytic cells and cytokines in infection and inflammation. The oxidative microbicidal superoxide-generating NADPH oxidase, the microbicidal granule proteins, and the chemoattractant receptors of phagocytic cells were studied.

Clinical Studies.

A LHD study completed two years ago demonstrating the efficacy of prophylactic γ-interferon to reduce infection frequency in patients with chronic granulomatous diseases (CGD) entered the second year of a phase IV study. This phase IV study is aimed at determining the long term safety for the patients and the continued effect of interferon-γ on both phagocytic cells and the inflammatory process. This treatment appears to be without adverse effects and patients continue to benefit from interferon-γ. A double-blinded randomized trial was continued to determine whether prophylactic oral itraconazole can reduce fungal infections in CGD. In related studies on the patient group with CGD, data were collated and published relating to infections with unusual fungal organisms.

Studies of patients with Mycobacterium avium intracellulare (MAI) have been expanded. A family group with X-linked susceptibility to MAI was identified, with a possible lymphocyte defect. A protocol is in progress which is examining the use of interferon-γ to enhance host defense against MAI in this patient group. To date seven patients have been studied. These studies have been very promising with 6 patients showing dramatic benefit from the interferon-γ therapy.

Studies of the use of interferon-γ in altering the humoral and cellular defects seen in HyperIgE-recurrent infection syndrome (HIE) are also in progress. This and other studies of use of interferon-γ in treatment of MAI and CGD noted above will yield important information about the use of this cytokine in various syndromes in which patients are prone to infection.

Clinical studies of normal volunteers and patients were continued to determine the systemic and local changes in cytokines and phagocytic cells in response to both local inflammation, modelled by experimental skin blisters, and systemic responses modeled by reaction to intravenous bacterial endotoxin. The time course for the accumulation of several cytokines and other mediators was documented. C5a, LTB4 IL1 and IL-8 appeared within 3 h in blisters; IL-6, G-CSF, and TNF-α appeared from 8-12 h. Following IV endotoxin TNF-α, IL-8, IL-6 and IL-1r antagonist appeared within 4 h while GCSF appeared after 8 h. Patients with HIE had a ten-fold normal increase in tumor necrosis factor in local lesions yet showed normal systemic responses to endotoxin. High levels of tumor necrosis factor in pulmonary tissues of HIE patients during staphylococcal pneumonia might cause the extensive tissue damage to lungs seen following infection in this disease. In related studies, administration of endotoxin to normal volunteers was associated with four-fold increases in circulating hematopoietic progenitor cells. This may reflect an important role for circulating progenitors in bacterial infections. Furthermore, endotoxin itself or the cytokines increased by endotoxin, such as IL-6, or G-CSF may be useful agents to enhance the yield of progenitor cells harvested from patients for gene therapy approaches to treatment as discussed below.

Studies of a patient with neutrophil specific granule deficiency demonstrated that this patient has a defect in expression of all eosinophil granule proteins except eosinophil peroxidase, but demonstrated increases of this defective eosinophil population in response to administration of granulocyte-macrophage colony stimulating factor. This demonstrates that the lesion of neutrophil specific granule deficiency extends to a defect of eosinophil granule protein production, but that actual production of these cells is unaffected. These studies demonstrate that there is a genetic regulatory element or elements common to both eosinophil and neutrophil granule development.

Pre-clinical studies of the development of new treatments

Studies have been initiated to develop methods of gene transfer of normal genes for the phagocyte NADPH oxidase into hematopoietic progenitor cells of CGD patients defective in such genes. These studies are aimed at gene therapy for CGD. Defective retroviruses free of helper virus activity were developed which were capable of transferring expression of normal oxidase genes in CGD cells defective in such genes. Transfer was associated with new protein expression and in related studies up to 10% of cells (both Epstein-barr virus transformed B-lymphocyte lines from CGD patients as one model and peripheral blood progenitor cell cultures as a second model) demonstrated correction of superoxide-generating capacity. These are important first steps in development of gene therapy for CGD and are model systems applicable to other genetic diseases of blood cells.

Basic science studies of phagocytic cells

Studies of the growth of peripheral blood progenitor cells in culture demonstrate that stem cell factor (c-kit ligand) greatly enhances proliferation of such cells, but acts only as a co-factor in the presence of other hematopoietic factors. In related studies it was demonstrated that in normal individuals the number of circulating blood progenitor cells is very similar suggesting that this is a highly regulated physiologic phenomenon. These observations are important for pre-clinical studies indicated above aimed at harvesting and targeting peripheral blood progenitors for development of gene therapy for CGD.

Recently, in our laboratory all of the protein components of the phagocyte superoxide-generating NADPH oxidase have been identified. The cytosolic factors include the previously identified p47phox and p67phox, while the third required cytosol factor was shown to be the low molecular weight GTPase, rac2. Rac2 associates with and may be regulated by a guanine nucleotide exchange inhibitor protein. In related studies the only membrane component of the oxidase was shown to be cytochrome b558. These studies also demonstrated that this protein is actually a flavocytochrome containing both a flavin and NADPH binding domain in its large subunit. In related studies, deletion mutant forms of the p67phox protein were created demonstrating that the catalytic elements reside in the amino terminal 28 kilodaltons. Recently, LHD investigators have pursued a role for src homology domains in the p47phox and p67phox elements of phagocytic cell NADPH oxidase. Studies in which point mutations of the genes for these proteins are produced and then inserted into B cells from CGD patients demonstrated a role for src domain interaction with proline rich sequences in the translocation of the NADPH cytosolic components to the membrane during oxidase assembly.

Studies of the expression of eosinophil granule proteins as recombinant proteins in E. coli have allowed the definition of sequence elements in these proteins which target the binding of a foldase (chaparone protein) groEL. These studies are likely to yield information about homologous mammalian chaparone proteins that may be involved in phagocytic cell granule protein synthesis. This past year a chaparone-like protein has been identified in phagocyte precursor cells and the importance of this protein is under study.

Preliminary work has been accomplished toward the end of creating a murine gene knock-out model of CGD. The cDNAs and genomic DNAs encoding the murine homologues of the human NADPH oxidase proteins defective in CGD have been cloned. These clones will be used for homologous recombination to inactivate the murine genes in embryonic stem cells. This animal model will be useful in the study of inflammation, the role of oxidants in host defense and as a model system for the gene therapy project outlined above.

Studies of chemotactic receptors of human phagocytic cells has been extended. Previously LHD scientists established the primary structure and signaling properties of the human N-formyl peptide receptor (FPR), the FPRL1 receptor (FPRL= formyl peptide receptor like), the FPRL2 receptor and interleukin-8 receptor B (IL8RB) from cDNA and gene cloning. LHD scientists have obtained the complete DNA sequence and structural organization of the human FPR gene, including an analysis of its putative promoter sequence, and have proposed a mechanism for the molecular evolution of the FPR gene family. In addition, characterization of the genes for IL8RA, IL8RB and the IL8RB pseudogene have been extended. All of these genes lack introns in the coding block but have large introns separating the start points for transcription and translation. Furthermore, the 5'-untranslated region of the FPR gene resides on three alternatively spliced exons, a very unusual finding. The sequence, ligand specificity, partial gene structure, expression and chromosomal localization of the first known receptor for a β chemokine, the human MIP-1α/RANTES receptor, which may mediate activation of both myeloid and lymphoid cells as well as regulate the proliferation of hematopoietic progenitors, have been described. A major finding from this work is that a putative receptor encoded by open reading frame US28 of human cytomegalovirus is 33% identical to the MIP-1α/RANTES receptor. Thus, US28 may be an example of molecular mimicry by viral hijack of a host gene. It was also shown that proteins involved in host defense functions are highly and exceptionally divergent between human and rodent species. A major new hypothesis was proposed to account for this phenomenon that has to do with molecular mimicry by acquisition of host genes by species-specific pathogens. New insights were also established regarding the molecular evolution of the ligand-receptor relationship.

Administrative, Organizational, and Other Changes.

Dr. John I. Gallin continues as the Chief and Dr. Harry L. Malech as the Assistant Chief of the Laboratory. Dr. Murphy is now a tenured scientist in the Laboratory. There are no Sections. Drs. Thomas Leto, Helene Rosenberg and Steve Holland are tenure track scientists in the laboratory.

Honors, Awards and Scientific Recognition

Dr. John I. Gallin was promoted to Assistant Surgeon General U.S. Public Health Service. Dr. Gallin serves as President of the International Society for the Immunocompromised Host. He continues to serve on the editorial Board of numerous journals and was invited to serve as visiting professor and to give numerous plenary lectures at major meetings in FY'93. He was invited to serve as on the Advisory Board of the Immune Deficiency Foundation and to serve as a special consultant to the President and Dean, Mt. Sinai Medical School.

Dr. Harry L. Malech was elected this year to the Association of American Physicians. Dr. Malech was appointed Coordinator of Gene Transfer Technology for NIAID Division of Intramural Research and also appointed as an Ad Hoc Reviewer, US Food and Drug Administration. Dr. Malech was invited as visiting professor and speaker at Medical Grand Rounds on July 23, 1992 at the University of North Carolina, Chapel Hill, NC. He was an invited speaker at the International meeting of the Japanese Association for Animal Cell Technology, Symposium on Gene Transfer Technology, November 30-December 4, 1992, Tokyo and an invited speaker at the 1993 Gordon Research Conference on Phagocytes, Plymouth, NH.

Dr. Philip M. Murphy was granted tenure at NIAID in FY'93 and he was invited to consult and lecture at major medical institutions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-18 LHD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies of Abnormal Host Defense

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
Others:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
	Steven M. Holland, M.D.	Senior Staff Fellow	LHD/NIAID
	Ellen DeCarlo, R.N.	Research Nurse	LHD/NIAID

COOPERATING UNITS *(if any)*

Douglas Kuhns, PRI-Frederick Cancer Research Facility, Julia Lekstrom, LCI/NIAID, K.J. Kwon-Chung, LCI/NIAID, Harvey I. Pass, Surgery Branch/NCI, McClellan M. Walther- Surgery Branch/NCI, Genentech, Inc.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
1.7		0.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of these studies was to study patients with abnormal host defense. In FY '93 studies focused on patients with abnormal phagocyte function. Particular emphasis was given to patients with chronic granulomatous diseases of childhood (CGD), who continue to be the subject of intense study. Other diseases that were studied include the Hyperimmunoglobulin-E recurrent infection (Job's) syndrome, neutrophil specific granule deficiency, leukocyte adhesion deficiency and other patients with recurrent infections who do not fall into a specifically defined disease category. Clinical investigations included: (a) description of a patient with "leaky leukocyte syndrome," a new disorder characterized by pyoderma gangrenosum in which circulating neutrophils spontaneous release enormous amounts of specific granule contents into the plasma (b) discovery of a patient whith chronic infection with atypical mycobacteria who has absence of a subpopulation of neutrophils that bind the monoclonal antibody C10, and (c) continuation of a phase 4 study evaluating long term effects of interferon-γ in CGD. We continue to monitor and expand these cohorts of patients who serve as models for long term studies of the immunological manipulation of the abnormal host defenses and for studies developing gene therapy of immune deficiencies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00481-08 LHD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Phagocyte NADPH Oxidase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Daniel Rotrosen, M.D.	Medical Officer	LHD/NIAID
Others: Choh L. Yeung, B.S.	Biologist	LHD/NIAID
Harry L. Malech, M.D.	Asst. Chief, LHD	LHD/NIAID
John I. Gallin, M.D.	Chief, LHD	LHD/NIAID

COOPERATING UNITS *(if any)*

Yoichi Osawa, Ph.D., LCP, NHLBI

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project explores the structure and biochemistry of the phagocyte NADPH oxidase, the redox chain responsible for production of microbicidal oxidants. A basic understanding of the biochemistry of this oxidase is critical to longterm efforts aimed at gene therapy for chronic granulomatous disease (CGD), and for development of novel agents to modulate the inflammatory response.

We have produced a recombinant flavocytochrome b558 (an oligomer of gp91phox and p22phox subunits) that for the first time enables reconstitution of NADPH oxidase in vitro solely from recombinant proteins. This observation is important for several reasons: a) it identifies unequivocally the essential protein components of the oxidase (flavocytochrome b558, p47phox, p67phox, and rac1 or rac2 GTPases); b) it demonstrates the feasibility of gene therapy for flavocytochrome b558-deficient forms of CGD using recombinant constructs derived from the same gp91phox and p22phox cDNAs; and c) it provides a model system for evaluating structure-function relationships and the significance of specific amino acid substitutions in patients with CGD due to a defective flavocytochrome b558.

Using oligopeptides modeled from gp91phox as candidate inhibitors of oxidase assembly, we identified regions of the integral membrane gp91phox (residues 35-48, 287-299, and 304-318) which appear to be cytoplasmic contact sites for p47phox. Thus, these gp91phox domains are analogous to the cytoplasmic COOH-terminal domain of gp91phox (amino acid residues 559-565) previously identified as a membrane docking site for p47phox. Through a similar approach we identified a domain of p47phox (amino acids YRRNSVRF, residues 324-331) that is essential for early interactions of p47phox and flavocytochrome b558. Further analysis suggests that the NH2 and COOH-terminal aromatic residues within this p47phox sequence play an essential role in activation of the oxidase. Complex formation with p47phox was shown to protect the flavocytochrome b558 heme prosthetic groups against destruction by amphipathic detergents. In the absence of p47phox these agents lead to heme destruction, release, and covalent adduct formation between heme degradation products and apoflavocytochrome b558. Hence, p47phox appears capable of cross-linking multiple domains of flavocytochrome b558 and these interactions may contribute to electron transfer by inducing conformational changes in the redox domains of flavocytochrome b558.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00521-06 LHD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Cytokines in Host Defense and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.,	Laboratory Chief	LHD/NIAID
Others:	Ellen DeCarlo, R.N.	Research Nurse	LHD/NIAID

COOPERATING UNITS *(if any)*

D Kuhns, Ph.D. PRI-Frederick Cancer Research Facility

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS.	PROFESSIONAL	OTHER
2.1		0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The kinetics of the appearance of cytokines and mediators of inflammation was determined in normal subjects and in selected patients with abnormal host defense. For this study a model of inflammation in the skin was employed using a blister device. Previously we showed that in normal subjects C5a and LTB4 appeared within 3 h of raising blisters, Il-8 and Il-6 appeared from 8 through 24 h and IL1-B, GM-CSF and TNF-α appeared from 12 to 24 h. Recently we have shown that "resting" exudate neutrophils are capable of secreting about ten times more Il-8 than "resting" peripheral blood neutrophils. When peripheral blood neutrophils are stimulated, synthesis of Il-8 is induced and Il-8 is stored in a membrane associated compartment that is readily mobilized and secreted. Exudate neutrophils also produce Il-6 and TNF-α. Studies in patients with chronic granulomatous disease revealed normal mediator accumulation in these patients. In contrast, patients with the hyperimmunoglobulin E-recurrent infection (HIE) syndrome had about ten-fold increased TNF-α accumulation in the blister fluid. The increased TNF-α was restricted to the local inflammatory response since in related studies, in which a preparation of the lipid A component of E. coli endotoxin was administered intravenously to normal volunteers and HIE subjects, normal increases in TNF-α, Il-8 and Il-6 in the circulation in HIE subjects was observed. Abnormal regulation of TNF-α at local inflammatory sites may have important implications in the pathogenesis of the post infectious complications seen in HIE such as bronchiectasis and bronchopleural fistula formation following pneumonia. In related studies, intravenous endotoxin was administered to normal volunteers and the kinetics of mediator production was followed in the subjects' sera. Increased Il-8 and TNF-α was temporally associated with the neutropenic and febrile phases of the response; increased soluble TNF-α receptor paralled increased total circulating TNF-α. Increases in Il-6 and G-CSF and small increases in GM-CSF correlated with the neutrophilia and increased lactoferrin observed following intravenous endotoxin. Changes in plasma Il-1, Il-2 and Il-4 were not detected following intravenous endotoxin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00614-03

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Phagocyte Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas L. Leto, Ph.D.	Senior Staff Fellow	LHD/NIAID
Others:	Isabelle Ossona de Mendez, Ph.D.	Visiting Fellow	LHD/NIAID
	Cheung H. Kwong, M.D., Ph.D.	Clinical Associate	LHD/NIAID
	Anthony G. Adams, B.A.	Biologist	LHD/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
3.6		

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is exploring the structure and function of important phagocytic cell proteins. Current efforts are focused on the components of the NADPH oxidase, an important host defense system that produces superoxide anion and related microbicidal oxidants. The cDNAs encoding all five known oxidase components have been expressed in several recombinant DNA expression systems (bacterial, insect and mammalian) enabling systematic manipulation of their structures and identification of relevant functional domains. Oxidase activity was restored to B-cells from patients with chronic granulomatous disease (CGD) by transfection with episomal expression vectors. Other more efficiently transfected model cell lines were also explored, including K562 cells, which produced superoxide when transfected with all four CGD genes together. Structural features critical to oxidase regulation by the fifth, ras-related G-protein, oxidase component (rac) have also been explored by comparing activities of chimeric recombinant proteins which have incorporated sequences from inactive ras homologs. The role of conserved src homology domains (SH3), found in p47-phox and p67-phox, on oxidase assembly and activation has been studied extensively. Comparison of the effects of deletions of these domains in both cell-free and intact transfected cell assays indicated a critical role in the membrane translocation of these proteins. Three proline-rich sites which bind to the src homology domains were identified within p47-phox and the small subunit of the transmembrane cytochrome b558 (p22-phox). A single proline mutation within p22-phox, observed in one form of CGD, was shown to abolish binding to the src homology domains of p47-phox. Competitive peptide binding studies suggest that switching from intramolecular to intermolecular interactions with the src-like domains in p47-phox enables its association with the membrane bound cytochrome during translocation. The target sites for SH3 domains in the oxidase system conform well with a consensus of other SH3 binding sequences defined in other systems (abl and GRB2, a mediator of EGF to ras signalling). Thus, these findings provide insights on a variety of signal transduction cascades which utilize this same conserved protein motif. These studies may aid in the design of agents which modulate the activity of this important inflammatory process and may facilitate development of gene therapy for CGD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00615-03 LHD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Neutrophil Activation by Chemoattractants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Philip M. Murphy, M. D.	Senior Investigator	LHD/NIAID
Others:	Ji-Liang Gao, Ph. D.	Visiting Associate	LHD/NIAID
	Sunil Ahuja, M. D.	Visiting Associate	LHD/NIAID
	H. Lee Tiffany, M. S.	Biologist	LHD/NIAID
	David McDermott, M. D.	Special Volunteer	LHD/NIAID

COOPERATING UNITS *(if any)*

Uta Francke, M. D., HHMI, Stanford University School of Medicine
Douglas Kuhns, Ph. D., PRI/Dyncorp, NCI-FRDC

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously established the 1° structure and signaling properties of the human N-formyl peptide receptor (FPR), the FPRL1 receptor (FPRL= formyl peptide receptor like), the FPRL2 receptor and interleukin-8 receptor B (IL8RB) from cDNA and gene cloning. We have now defined the complete DNA sequence and structural organization of the human FPR gene including an analysis of its putative promoter sequence, and have proposed a mechanism for the molecular evolution of the FPR gene family. We have also extended our partial characterization of the genes for IL8RA, IL8RB and the IL8RB pseudogene. All of these genes lack introns in the coding block but have large introns separating the start points for transcription and translation. Furthermore, the 5'-untranslated region of the FPR gene resides on three alternatively spliced exons, a very unusual finding. We have also defined the sequence, ligand specificity, partial gene structure, expression and chromosomal localization of the first known receptor for a β chemokine, the human MIP-1α/RANTES receptor which may mediate activation of both myeloid and lymphoid cells as well as regulate the proliferation of hematopoietic progenitors. A major finding from this work is that a putative receptor encoded by open reading frame US28 of human cytomegalovirus is 33% identical to the MIP-1α/RANTES receptor. Thus US28 may be an example of molecular mimicry by viral hijack of a host gene. We have shown that proteins involved in host defense functions are highly and exceptionally divergent between human and rodent species. A major new hypothesis was proposed to account for this phenomenon that has to do with molecular mimicry by acquisition of host genes by species-specific pathogens. New insights were also established regarding the molecular evolution of the ligand-receptor relationship.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00644-02

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of peripheral blood progenitors: a target for gene transfer.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
Others:			
	Sudhir Sekhsaria, M.D.	Clinical Associate	LHD/NIAID
	Fei Li, Ph.D.	Visiting Fellow	LHD/NIAID
	Meir Shalit, M.D.	Visiting Scientist	LHD/NIAID
	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID

COOPERATING UNITS *(if any)*

Thomas Nutman, M.D., LPD, NIAID

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies peripheral blood progenitors (PBP) as a target for retrovirus mediated gene therapy of inherited diseases affecting the function of human phagocytic cells, including neutrophils, eosinophils, and monocytes. Studies of growth factors which influence the differentiation of PBPs are also a part of this project. PBPs which may include totipotent stem cells are similar or identical to the blood precursors in bone marrow, but circulate in small numbers in peripheral blood. PBP harvested and concentrated from peripheral blood by centrifugation apheresis have been used by others as an autologous transplant in lieu of or combined with progenitors harvested from bone marrow to reconstitute the bone marrow of patients with cancer treated with cytotoxic chemotherapy. PBP harvested from patients with inherited immune diseases such as chronic granulomatous disease or other immune deficiency are a readily available target for functional correction by gene transfer. We purified PBP from apheresis derived leukocytes and studied conditions for differentiating them into neutrophils, monocytes, and/or eosinophils using both liquid and soft agarose cultures. In one series of studies we demonstrated that stem cell factor (also called c-kit ligand) was not active alone, but when combined with other hematopoietic factors greatly increased both the number and size of myeloid colonies derived from PBP. In related studies we demonstrated that the eosinophil factor IL5 alone would not support colony growth of PBPs in the early stages of differentiation, but that IL5 alone would support colony growth after culturing PBPs for 4 days in early acting growth factors. This suggests that responsiveness to late acting hematopoietic factors may require the expression of receptors to such factors. This type of information is essential to the goal of proliferation of hematopoietic progenitors in vitro for purposes of gene therapy or transplantation. Studies of the number of circulating PBP indicated that this is a highly regulated and constant parameter, but that endotoxin administration, a model of gram negative bacterial infection, increased the number of circulating PBP five-fold. In other studies using the retroviral constructs we determined optimum conditions for transfer of genes into PBP. Determination of such conditions are essential to the development of protocols for gene therapy for immune system disorders affecting bone marrow.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00645-02

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Gene therapy for immune deficiencies.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
CoPI:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
Others:			
	Sudhir Sekhsaria, M.D.	Clinical Associate	LHD/NIAID
	Fei Li, Ph.D.	Visiting Fellow	LHD/NIAID
	Michael Mardiney, M.D.	Clinical Associate	LHD/NIAID

COOPERATING UNITS *(if any)*

Eli Eisenstein, LCI, NIAID; R. Michael Blaese, NCI;
Richard Mulligan, Ph.D., Whitehead Institute.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This project studies gene therapy for chronic granulomatous diseases (CGD) and other inherited immune diseases affecting human phagocytes. CGD are a group of 4 distinct genetic disorders with common phenotype characterized by life-threatening recurrent infections caused by failure of blood neutrophils and monocytes to produce superoxide and hydrogen peroxide. CGD results from the failure to produce any of the components of the NADPH oxidase required for superoxide generation. We have now successfully used retrovirus mediated gene transfer techniques to functionally correct 3 forms of CGD, the autosomal $p47^{phox}$ deficient CGD and the X-linked and autosomal forms of CGD involving abnormalities of flavocytochrome b558 subunits $gp91^{phox}$ and $p22^{phox}$. We have developed two *in vitro* model systems to demonstrate the feasibility of genetic correction of CGD. The first system uses Epstein-Barr virus transformed B-lymphocyte cell lines (EBV-BL). EBV-BL from normal individuals produce small amounts of superoxide when stimulated, while those derived from CGD patients do not produce superoxide and are missing the oxidase component characteristic of that genetic form of CGD. The retrovirus vector, MFG, which produces high viral titers and higher protein production was used to transduce $p47^{phox}$, $gp91^{phox}$ and $p22^{phox}$ cDNAs into EBV-BL to correct the CGD defect routinely obtaining a 1 to 15% correction rate without selection for transduced cells. This trait was stable for months of subsequent culture. Correction was specific to transduction of the defective component cDNA. In the second model system, marrow-like myeloid progenitor cells harvested from the peripheral blood could be induced to proliferate and differentiate *in vitro* to mature neutrophils capable of superoxide production. Similar cells from CGD patients could not produce superoxide, but transduction of these precursors from CGD patients with the MFG retrovirus containing the normal version of the specific CGD gene resulted in correction of superoxide production in 4-20% of resultant neutrophils. Theoretically, the peripheral blood progenitor model system of gene transfer could be scaled up to a procedure capable of being used for *in vivo* gene therapy for immune system and other disorders affecting bone marrow cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00646-02

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transgenic Animal Models of Human Immune Defects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Steven M. Holland, MD	Senior Staff Fellow	LHD/NIAID
Others:	Sharon H. Jackson, MD	Clinical Associate	LHD/NIAID
	Daniel Rotrosen, MD	Senior Staff Fellow	LHD/NIAID
	John I. Gallin, MD	Laboratory Chief	LHD/NIAID
	Eli Eisenstein, MD	Clinical Associate	LCI/NIAID
	Warren Strober, MD	Section Chief	LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL.	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This project is directed at understanding the role of specific enzyme systems in generating and maintaining host immunity. The approach taken involves the creation of mice with discrete defects in host defenses by using homologous recombination of DNA into the host genome to disrupt relevant genes. Lesions will be introduced that either correspond to previously identified mutations in humans or that incapacitate systems probably involved in host defense. Therefore, this project relies upon a thorough understanding of the molecular and functional organization of the NADPH oxidase system and the mutations which disable it. Further, the dissection of specific phenotypes associated with defined genotypes may provide insight into the importance of certain functional domains of the genes of the NADPH oxidase and indicate which sites are most informative for further study.

We have used the NADPH oxidase system as a paradigm for the creation of mice with a genetic defect similar to one found in humans, chronic granulomatous disease (CGD). To this end, we have cloned, sequenced, and functionally expressed the cDNA of the murine homolog of the human $p47^{phox}$ gene. We have cloned, partially sequenced, characterized, and disrupted genomic fragments from both the p47phox and gp91phox genes in preparation for re-insertion into the mouse genome. Disruption of these genes will provide the bases for a murine model of the autosomal recessive and X-linked forms of CGD, respectively. Culture of the embryonal stem cells which will provide the targets necessary to this project is now established in the laboratory.

In the course of this work we have 1) identified two introns in the murine p47phox gene which are not found in the human gene, 2) characterized two novel mutations in a large family with CGD related granulomatous colitis, 3) identified and partially characterized a family with ineffective granulomatous inflammation as demonstrated by disseminated *Mycobacterium avium* infection, 4) overcome the inflammatory defect in the family with disseminated *Mycobacterium avium* infection by treatment with interferon gamma.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00647-02

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genes and Gene Products as Immuno-adjuvants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	John I. Gallin M.D.	Laboratory Chief	LHD/NIAID
	Sharon H. Jackson, M.D.	Clinical Associate	LHD/NIAID
	Angela M. Toms, B.A.	Medical Technologist	LHD/NIAID
	Helene F. Rosenberg, M.D., Ph.D.	Senior Staff Fellow	LHD/NIAID

COOPERATING UNITS *(if any)*

Thomas C. Quinn, M.D.	Senior Investigator	LIR/NIAID

LAB/BRANCH

Laboratory of Host Defenses

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at understanding, characterizing, and evaluating a novel form of gene product delivery for immunization and treatment: direct DNA injection. Cytokines will be administered intramuscularly to mice in the form of either a cDNA expression vector or preformed protein simultaneously or in sequence with cDNAs for specific antigens from chlamydia in order to elicit a strong immune response. Animals will be followed by evaluation of cytokine response in the circulation, local response to the injected materials at the site of administration, and development and type of immunity. To these ends we have obtained and/or engineered into expressible forms the cDNAs for murine TNF-alpha, murine IL-2, murine IL-4, and murine IL-6. In order to develop the source for the chlamydial antigens needed for these studies, we have generated DNA libraries in a bacterial plasmid system from both *Chlamydia trachomatis* and *Chlamydia pneumoniae*. From these libraries we have cloned the genes for the chlamydial major outer membrane proteins and the chlamydial 57kd heat shock protein. These genes have been transferred into bacterial and eukaryotic expression systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00648-03

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NADPH Oxidase Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Anthony G. Adams, B.A.	Biologist	LHD/NIAID
	Harry L. Malech	Assistant Lab Chief	LHD/NIAID
	John I. Gallin	Laboratory Chief	LHD/NIAID

COOPERATING UNITS *(if any)*

Richard T. Kenney, M.D., LPD, NIAID
Hiroyuki Nunoi, M.D., Dept. of Medicine, Kumamoto University, Kumamoto, Japan

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4		

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☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is exploring the organization of NADPH oxiase genes. This enzyme is responsible for production of superoxide and related microbicidal oxidants. Deficiencies in this host defense system result in chronic granulomatous disease (CGD), which is characterized by serious recurrent infections and excess granuloma formation. This program will explore genetic lesions that affect NADPH oxidase activity in CGD, provide gene mapping information enabling diagnosis of CGD, and suggest strategies for correction of these gene defects. Current efforts are focused on the gene encoding the p67-phox oxidase component. Genomic clones from this locus have been extensively characterized with respect to all exon-intron boundaries and their flanking sequences. This information has enabled design of oligonucleotide primers needed for PCR analysis of CGD lesions using small amounts of genomic DNA from any tissue source. Using this method, a 20 basepair deletion on the 3′ boundary of exon 5 was noted in one p67-phox deficient patient. The corresponding cDNA from this patient was cloned to define the effects of this abberrant splice site, which likely resulted from an uneven crossover event. Several other p67-phox deficient patients have been identified and several structural variations (deletions, point mutations, and insertions) have been observed. A related project (Z01 AI 00614) has established the means for stable gene transfection and oxidase restoration using episomal vectors that endow hygromycin resistance. The effects of these putative CGD gene defects on NADPH oxidase activity will be explored with this gene transfer system. We are developing this gene transfer method to produce stable "model CGD lines", which express three of the four oxidase components and will enable functional analysis of lesions in any of the four CGD genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00649-02

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Phagocyte Granule Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Helene F. Rosenberg, M.D., Ph.D.	Senior Staff Fellow	LHD/NIAID
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COOPERATING UNITS (if any)

Laboratory of Intracellular Parasites, RML, NIAID (R. Morrison), Graduate Genetics Department, George Washington University (H. Lee Tiffany)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to determine the molecular events involved in the biosynthesis and activation of eosinophil and neutrophil granule proteins. Studies in progress focus on A. granule protein folding, B. specific granule deficiency, and C. released granule proteins

A. Protein folding. We have described the high affinity, reversible interaction of the prokaryotic molecular chaperone, groEL, with nascent chains of two eosinophil granule proteins (Rosenberg, HF et. al. J. Biol. Chem. 268: 4499-4503 (1993)). Using a panel of monoclonal antibodies, we have identified a novel protein present in early myeloid progenitors that shares at least one epitope with groEL. This protein is not present in mature peripheral blood cells. Further characterization of this protein is in progress.

B. Specific granule deficiency. We have shown that the disorder known as neutrophil specific granule deficiency also includes the eosinophil lineage (Rosenberg HF and Gallin JI. Blood 82:268-273 (1993). We have shown that three of four eosinophil granule proteins are absent from peripheral blood cells from a patient with neutrophils specific granule deficiency.

C. Released granule proteins. Using monoclonal antibodies that distinguish between storage and secreted form of an eosinophil granule protein, we have results suggesting that the released protein is deglycosylated relative to the storage form. The molecular events yielding this deglycosylation are under study.

PHS 6040 (Rev. 5/92)

LABORATORY OF IMMUNOLOGY
1993 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1992 through September 30, 1993

Introduction

The goals of the Laboratory of Immunology (LI) are the elucidation of the fundamental mechanisms of the immune system; the application of the resultant knowledge to the understanding of the pathogenesis of immunologic disorders such as autoimmunity, immunodeficiency, and allergic diseases; and the development of new approaches to immunization that would be useful in vaccine development. LI scientists have made important progress toward these ends through the application of powerful new technologies that have revolutionized modern biomedical science. These include techniques of contemporary molecular biology, preparation and study of transgenic mice, development and use of monoclonal antibodies, cloning of normal and transformed lymphocytes, and flow cytometric analysis and sorting. These methods combined with new techniques in protein chemistry and cell biology have allowed the solution of many of the major problems in immunology and should lead to important advances in efforts to prevent and treat diseases of the immune system and disorders caused by the action of immune cells and antibodies.

The X-Linked Immunodeficiency of Mice is Caused by a Point Mutation in the Gene Responsible for X-Linked Agammaglobulinemia in Humans

The x-linked immunodeficiency (*xid*) gene of mice results in an inability to make antibody responses to polysaccharide antigens, striking diminution in serum IgM and IgG3 levels and reduction of B cells number to ~1/2 of normal. On the other hand, the responses of *xid* mice to protein antigens are relatively normal and no abnormality of T cell function has been noted. Based on reconstitution experiments and analysis of X-chromosomal inactivation in heterozygotes, it is clear that the *xid* defect is expressed in the B cell compartment. In order to determine the genetic basis of this abnormality, LI scientists undertook a positional cloning effort. Mice bearing the *xid* gene (CBA/N mice) were mated to a strain (Mus musculus Skive)that was genetically quite distinct from conventional laboratory strains. The resulting F1 females were mated to CBA/N males. More than 1100 progeny were analyzed for inheritance of the *xid* gene from their mothers and for their inheritance of closely linked genetic markers. Through this analysis, it was found that the simple sequence repeat marker *DXMit3* was within 0.1 cM of *xid.* Yeast artificial chromosomes (YACs) were obtained that bore *DXMit3*. At that stage, a candidate gene was suggested based on the cloning of the gene for the human disease X-linked agammaglobulinemia (Xla). This gene, *Bruton's agammaglobulinemia tyrosine kinase* (*btk*), has a homologous location on the human X chromosome to that of *xid* on the mouse X chromosome. All *DXMit3* positive YACs contained the mouse *btk* gene. A genetic polymorphism in the *btk* gene allowed it to be mapped in relationship to *xid.* No recombinants in the entire backcross between *xid* and *btk* were found. Sequencing the *btk* cDNA derived from CBA/N mice and the closely related normal strain CBA/CaHN revealed a single nucleotide difference resulting in a change at position 28 from arginine to cysteine. This mutation appeared significant because in seven mammalian species, this position in *btk* was always an arginine. Indeed, the sequence immediately surrounding this position is exceedingly highly conserved. This mutation is in the unique region of the kinase. It does not alter the autophosphorylating activity of *btk* nor does it effect its degree of expression. Current views are that it may effect the subcellular localization of *btk* or its interaction with various substrates. Interesting, these results suggest that *btk* functions throughout B cell development.

Deletion of *btk* or disabling its kinase activity cause the profound defect represented by Xla in humans while more subtle defects in B cell function can be observed with the point mutation represented by *xi*d. It seems likely that human defects in B cell function caused by *xid*-like point mutations exist and may responsible for less severe abnormalities that Xla (J.D. Thomas and W.E. Paul, LI/NIAID; P. Sideras, Univ. of Umea; I. Vorechovsky and C.I.E. Smith, Karolinska Institute; V. Chapman, Roswell Park Memorial Institute).

Diversification of Immunoglobulin Heavy Chain Variable Region Gene Sequences in the Rabbit Appendix

Although the rabbit immunoglobulin (Ig) H chain genetic region contains multiple variable (V_H) genes, 80 to 95% of the B cells of mature rabbits express an Ig that is derived from the same V_H gene (V_H 1). This suggests that rabbits, like birds, principally utilize somatic diversification methods that are distinct, at least in quantitative terms, from those employed by many other mammals. To examine this, LI scientists have studied the expression of the variable region of Ig H chains in individual follicles of the rabbit appendix. The appendix develops rapidly between birth and six weeks of age in the rabbit, when it reaches it maximal size. Within individual follicles of the appendix, light and dark zones may be distinguished by staining with the lectin SWGA. Small numbers of cells from both light and dark zones obtained with the use of a micromanipulator were analyzed for sequence using polymerase chain reaction. This has allowed an analysis of the means through which diversification is achieved. This analysis shows that the V_H1 gene may rearrange to several different D_H region genes. In individual follicles, there appeared to be substantially less diversity in the rearrangement to D_H than in the appendix as a whole. In the examination of diversification within V_H1 itself, cells in the light zone of the appendix follicles express sequences closer to the germline than do cells in the dark region. It appears likely that the changes observed represent gene conversion and/or somatic hypermutation occurring within the dark zone of appendix follicles resulting in striking V_H diversification. This process appears to be linked to antigen-mediated selection occurring as a result of trapping of antigen on the surfaces of follicular dendritic cells in close proximity to the cells in which V_H1 diversification is occurring. These studies should aid in developing a detailed understanding of the dynamic processes through which individual V_H genes may undergo diversification. The rabbit should allow the study of this process in detail since it principally uses only one V_H gene, thus reducing the difficulty of relating products to their germline ancestral gene (P.D. Weinstein and R.G. Mage, LI/NIAID; A.O. Anderson, USAMRID).

In Vitro Translation, Folding, Disulfide Bond Formation and Assembly of Class I MHC Molecules

The central function of class I MHC molecules is to bind peptides derived from antigens, to transport these to the cell surface, and to be co-recognized with the peptide by T cell receptors and CD8 molecules. A detailed understanding of the process through which class I molecules bind and fold around peptide will depend upon the ability to follow this process in vitro. To that end, LI scientists have developed a system in which the major steps of the biosynthetic process could be carried out in vitro. Full length cDNAs for H-2L^d, H-2K^d and β2-microglobulin were cloned into bacterial plasmids so that they could be transcribed in vitro with bacteriophage RNA polymerases. These homogeneous mRNAs were used to direct in vitro synthesis of the encoded proteins in a reticulocyte lysate/ canine pancreas microsome translation/ assembly system. Using this system, it was found that the proteins were translated and intrachain disulfide bonds formed. The α3 domain appeared to fold normally and to generate antigenic epitopes associated with the native state. In the absence of co-translated β2-microglobulin, few if any α1 or α2 domain epitopes were formed. If β2-microglobulin was co-translated with the class I heavy chain, some folding of α1 and α2 was observed, based on expression of structure-related epitopes. However, the greatest effect on folding was observed when a minimal antigenic peptide was added to the translation/folding system. Thus, with co-translation of class I heavy chain and β2-microglobulin and with the presence of a minimal peptide, the molecules quantitatively fold into their normal structure. In parallel with the formation of epitopes that identify the native structure, β2-microglobulin becomes more tightly associated with the rest of the complex. This system provides a tool for the detailed study of the folding process and should allow rapid analysis of the critical elements that regulate such folding by site-directed mutagenesis of the components. Further, the addition of other proteins that appear, from in vivo data, to participate in this system, such as TAP1 and TAP2, can be critically evaluated (R. Ribaudo and D.H. Margulies, LI/NIAID).

The Role of Invariant Chain in Intracellular Trafficking of Class II MHC Molecules

The invariant chain, associated intracellularly with class II MHC molecules, is believed to play an important role in the regulation of peptide loading, by binding to newly synthesized class II molecules and preventing peptides from binding to them in cellular compartments other than endosomes and lysosomes. However, LI scientists have found the antigen-presenting cells (APC) from mice, in which the invariant chain gene has been inactivated by targeted disruption, do not efficiently acquire peptides in the endoplasmic reticulum. This strongly suggests that invariant chain plays other critical roles in regulating class II function. Evidence has now been obtained to show that APC of invariant chain$^{\text{minus}}$ mice have a gross defect in the movement of class II molecules out of the endoplasmic reticulum. This has led to studies that demonstrate that invariant chain-associated class II molecules normally move from the endoplasmic reticulum to early endosomes. Further, the cytoplasmic tail of the invariant chain contains the targeting signals that transport the complex to early endosomes. Indeed, the presence of invariant chain has a major effect on the early endosomes themselves and appears to delay endosomal maturation. This delay in endosomal development may enhance mixing of newly synthesized class II molecules with peptides derived from recently endocytosed antigens so that they move to more mature endosomes in a coordinated manner thus increasing the likelihood of efficient loading of class II molecules. These results emphasize the importance of the invariant chain in distribution of class II molecules in subcellular compartments and, in particular, in their concentration in sites that provide optimal opportunities for peptide loading (P. Romagnoli and R.N. Germain, LI/NIAID; J. Yewdell, LVD/NIAID).

Binding of Peptide to Class II Molecules Stabilizes Them, Prevents Their Aggregation and Allows Them to be Transported to the Cell Surface

Class II MHC molecules bind peptides from endocytosed antigens; the resultant complex is transported to the cell surface where it can be recognized by CD4+ T cells with specific receptors and can induce specific responses by those cells. LI scientists have made important contributions to the understanding of the process through which class II molecules are loaded with peptide and of the mechanisms that lead to their transport to the cell surface or, alternatively, to their degradation. Biochemical studies of class II molecules reveal that two distinct phases of binding to peptide exist. There is a rapid initial interaction that is marked by a fast dissociation rate followed by slow process through which highly stable complexes are accumulated. This latter process is markedly enhanced at the acidic pH found in the endosomal compartment where peptide loading occurs. Furthermore, class II molecules that fail to bind peptide undergo rapid aggregation with other proteins under acidic conditions, presumably as a prelude to degradation. Recent studies using cells from mice that lack the invariant chain, normally found associated with non-peptide bound class II molecules, indicates that this chain protects non-peptide loaded class II molecules from aggregation. Thus it appears that as class II molecules are released from invariant chain under the acidic conditions of the endosome, they will either be targeted for aggregation or for transport to, and expression on, the cell surface depending on whether they bind peptide and assume a compact structure as a result of such binding. These studies give insight into the dynamics of the process through which class II molecules bind and present antigen and thus should be of importance in the design of optimally immunogenic vaccines and of inhibitors of T cell activation (S. Sadegh-Nasseri, A. Rinker, Jr., J. van Meerwijk, L.-Y. Huang and R.N. Germain, LI/NIAID; E.K. Bikoff and E.J. Robertson, Harvard University).

Direct Measurement of Binding of Soluble Class I MHC Molecules to Peptides by Surface Plasmon Resonance

Class I MHC molecules function by binding peptides derived in cytosolic compartments of the cell, transporting these peptides to the cell surface and presenting them to CD8+ T cells with complementary receptors. Although much progress has been made in understanding the binding of peptides to class I molecules, direct measurements of these interactions have been hampered by the difficulty of carrying out binding studies in solution. With the introduction of instruments that utilize surface plasmon resonance to measure mass bound to surfaces, an opportunity is presented for rapid and precise measurement of the interaction of peptides that have been immobilized on a solid phase with soluble class I molecules. LI scientists have pioneered in the use of surface plasmon resonance to measure this interaction. Through the development of techniques to bind peptides to the solid phase through cysteines, it was possible to evaluate the importance of each residue in a nine residue peptide for its contribution to binding to class I molecules by systemically preparing cysteine mutants at each position and using those cysteines to tether the peptide to the solid surface. Those mutations that interfere with binding are interpreted to reflect sites that normally participate in the interaction of peptide with class I molecules. Those that do not are candidates to participate in the interaction with the T cell receptor. A detailed analysis of the binding of a cytomegalovirus peptide to the H-2L^d class I molecule revealed that positions 2,3 and 9 were critical to the binding of the peptide to L^d and that position 1 made an important contribution. By contrast, coupling peptides through positions 4,5,6,7 or 8 did not interfere with peptide binding. This strategy allows the assignment of all residues of a peptide in terms of importance in class I binding. In addition, this technology allows careful kinetic analysis of the binding of MHC to peptide. Such studies reveal that the association rate is slow, 1000 to 5000 $M^{-1}sec^{-1}$, and that the dissociation rates are also very slow, in the range of 10^{-4} to 10^{-6} sec^{-1}, depending on the peptide and the class I molecule. Calculated equilibrium constants are 10^{-7} to 10^{-9}M.

Furthermore, these techniques allow the accumulation of a large body of data regarding the binding of peptides to class I molecules. In turn, molecular modeling of such interactions provides substantial insight into the binding and allows the derivation of binding "rules". Thus, in the binding of peptide to D^d, motif residues of the peptide act as anchors; further, the basic motif residue is in a position to form a salt bridge with aspartic acid156 of D^d, a residue that is highly polymorphic among class I molecules. This implies that this interaction contributes considerable specificity to the peptide/D^d interaction. These studies provide detailed insight into the control of interactions between antigen-derived peptides and class I MHC molecules. Such information will be of great value in the rational design of immunogens for vaccines and in efforts to induce tolerance to autoantigens, allergens and transplantation antigens (M. Corr, L.F. Boyd, S. Kozlowski, S. Khilko, A. Lees, J.K. Inman and D.H. Margulies, LI/NIAID; E.A. Padlan, LMB/NIDDK).

Molecular Anatomy of an HIV gp160 T Cell Epitope

In order to gain insight into the basic mechanisms underlying recognition of epitopes on gp160 of HIV by CD4+ T cells, LI scientists, working with colleagues in NCI, have focused their attention on a previously identified determinant (T1: residues 428-443) that elicits responses in mice. Such responses depend upon binding of the peptide representing this region of gp160 to the class II molecule I-E^k. A series of alanine-replacement peptides were constructed and tested for their ability to bind to I-E^k molecules and to elicit responses by a T cell hybridoma specific for I-E^k/gp160 peptide. This analysis revealed that only four residues derived from the original peptide were essential for binding to I-E^k, although this minimal peptide did not elicit a response by the gp160-specific T cell hybridoma. It was further shown that a glutamic acid in the peptide strongly diminished its capacity to be bound by I-E^k. Removing that glutamic acid resulted in a 30-100 fold increase in binding to I-E^k. Indeed, the use of the glutamic acid^{minus} variant of the peptide resulted in a stimulant that was at least 10-fold more potent that the natural determinant in the stimulation of T cells specific for the natural sequence. Furthermore, an alteration in the glutamic acid at position 29 of the E^b chain of I-E^k resulted in a striking increase in T cell responses to wildtype peptide bound to I-E^k. These data provide striking insights into the minimal requirements for peptide/class II interaction and also demonstrate the importance of interfering effects by non-essential residues in both the peptide and the class II molecule. They suggest strategies for improving the design of immunogens to be included in vaccines that may enhance potency without loss of specificity for the infectious agent (W.H. Boehncke, S. Sadegh-Nasseri, L. Racioppi and R.N. Germain, LI/NIAID; T. Takeshita and J.A. Berzofsky, MB/NCI).

Analysis of the IL-4 Promoter and of the Basis of Restricted IL-4 Expression in TH2 CD4+ T Cells

IL-4 is not produced by naive T cells but is produced by a subset of T cells that have differentiated down the "TH2" pathway. By contrast, T cells that have differentiated down the TH1 pathway fail to produce IL-4. In order to gain insight into the molecular basis of this process, LI scientists have undertaken an analysis of the IL-4 promoter. Two segments of the IL-4 promoter have been identified that are necessary for full phorbol ester-inducible activity in EL-4 cells. Deletional and mutational analysis has revealed that one of these segments is indispensable for promoter function. This sequence was designated consensus sequence 1 (CS-1), as it shares homology with four other sequences that occur within the 302 bp region 5' of the gene. CS-1, and the second sequence (CS-2), occur in an 83 bp construct that retained full promoter activity. Both CS-1- and CS-2-containing oligonucleotides bound to phorbol ester-inducible factors in electrophoretic mobility shift assays. The resulting complexes were slightly different in size and were specifically inhibited in binding only by the homologous oligonucleotide indicating that the factors were different. Indeed, multimer constructs of CS-1- and CS-2-containing oligonucleotides linked to a minimal promoter revealed that CS-1 had much more activity than CS-2. Most strikingly of all, the multimer CS-1 construct could direct transcription of the reporter gene transfected into an IL-4-producing T cell clone (D10) but not when transfected into a non IL-4 producing clone (A.E7). This implies that the CS-1 containing oligonucleotide and the factor(s) that bind to it play a critical role in the differentiated phenotype of TH2 and TH1 T cell clones. Efforts to determine how this distinctive regulation is achieved are now in progress. This should provide a molecular basis for the differentiation of the two principal subpopulations of CD4+ T cells (K. Bruhn, K. Nelms, W.E. Paul and M. Lenardo).

A Common Signalling Pathway for the IL-4 Receptor and the Insulin Receptor

IL-4 mediates its effects by binding to high affinity receptors expressed on target cells. The IL-4 receptor is a member of the hematopoietin receptor family. It cytosolic domain, however, is quite distinct from those of other members of the family. Addition of IL-4 to many IL-4 responsive cell populations results in rapid tyrosine phosphorylation of a 170 kDa substrate, designated 4PS, that is distinct from substrates phosphorylated in response to other hematopoietins. Stimulation of myeloid cell lines with insulin and insulin-like growth factor-1 (IGF-1) also leads to phosphorylation of 4PS. In fibroblasts, insulin and IGF-1 lead to the prompt phosphorylation of the substrate IRS-1. 4PS and IRS-1 appear to be distinct since their phosphopeptide maps are different. Antibodies to IRS-1 show some cross-reactivity with 4PS suggesting that they are related molecules. Hematopoietic cells such as FDC-P1 or 32D that express IRS-1 as a result of stable transfection will phosphorylate this substrate in response to IL-4, insulin or IGF-1, reinforcing the relatedness of 4PS and IRS-1. Both 4PS and IRS-1 become associated with the SH2-domain containing protein PI-3-kinase as a result of IL-4 stimulation. In order to study the requirements for receptor signalling in more detail, truncation mutants of the human IL-4 receptor were transfected into 32D cells that stably express IRS-1. The capacity of receptor mutants to transmit growth signals and IRS-1 phosphorylation were linked. In particular, deletion of the region between residues 437 and 557 destroyed both activities. A fusion protein containing this region was shown to be able to bind IRS-1 and also, in the presence of extracts from receptor expressing cells, to allow its phosphorylation, suggesting that this region can bind both IRS-1 and IL-4 receptor-associated kinases. A motif was recognized in this region of the IL-4 receptor homologous to a sequence surrounding a critical tyrosine in the insulin and IGF-1 receptors. This motif, termed the I4R motif, consists of PLXXXNPXYXSXSD. These results provide the first detailed picture of signal generation through the IL-4 receptor and shed considerable light on the means through which lymphokines mediate their biological effects (A.D. Keegan, K. Nelms and W.E. Paul, LI/NIAID; L.-M. Wang and J.H. Pierce, NCI; M.F. White, Joslin Diabetes Center).

Regulation of Transcription of the IL-2 Gene by Co-Stimulatory Molecules

One of the critical features of T cell responses to antigen-derived peptides presented by class II (or class I) molecules on antigen-presenting cells in the production of lymphokines such as IL-2. It has been shown that optimal induction of IL-2 production requires that, in addition to engagement of the T cell receptor by peptide/class II, co-stimulatory signals provided by antigen-presenting cells are important. Of these, the best understood is the interaction between B7 and CD28. Indeed, the stimulation of primed T cells in the absence of co-stimulatory signals not only fails to induce lymphokine production but may also lead to an unresponsive or anergic state in which these cells fail to produce IL-2 upon restimulation with both peptide/class I and co-stimulators. LI scientists have attempted to understand the molecular basis of co-stimulation. A set of stably transfected T cell clones have been generated to allow the analysis of critical regulatory elements in the IL-2 promoter that mediate the effect of the co-stimulatory molecules. In a system that takes advantage of fibroblast antigen-presenting cells and co-stimulatory signals provided either by introducing co-stimulants into them by transfection or by using antibodies to cross-link co-stimulatory receptors on the T cell, it has been shown that factors that bind to both the κB and AP-1 elements of the promoter respond to co-stimulants. This was particularly interesting in view of earlier data that had shown that the induction of clonal anergy was associated with a diminution of induction of factors that interact with AP-1 and to a lesser extent with κB. By contrast, the NF-AT and octamer binding regions of the IL-2 promoter were unaffected by the co-stimulatory process. These studies provide insight into how co-stimulation at the cell surface is mediated and may lead to approaches to enhance or to diminish such accessory cell determined events (L. Zheng and M.J. Lenardo, LI/NIAID).

Interleukin-10 Inhibits Macrophage Co-Stimulatory Activity by Blocking the Induction of B7

Interleukin-10 (IL-10) is a potent cytokine produced by T cells, macrophages and B cells. It has striking effects in diminishing production of interleukin-2 (IL-2) and interferon gamma (IFNγ) by T cells. Evidence has been obtained by LI scientists and others that IL-10 mediates this effect by acting on the antigen-presenting cell (APC) rather than the responding T cell. Moreover, IL-10 only inhibits IL-2 and IFNγ production when the major APC in the system is a macrophage. To gain insight into the mechanism through which IL-10 mediates this inhibitory activity, LI scientists have devised a system in which then direct action of IL-10 on macrophages was studied in the course of their in vitro activation. Attention was focused on the induction of the potent membrane associated co-stimulator, B7. B7 acts as a ligand for CD28 and CTLA-4, receptors expressed on T cells. The B7/CD28 interaction has a potent co-stimulatory activity that striking increases lymphokine production by T cells. Freshly isolated, non activated macrophages express little or no B7; in the course of their activation in vitro and in vivo, such as would occur in the course of infections with intracellular microorganisms, B7 is induced on the macrophage, thus strikingly increasing its competence as an APC. During the past year, LI scientists have shown that IL-10 prevents the induction of B7 on macrophages and have further shown that such IL-10-treated macrophages can stimulate T cells to produce IL-2 and IFNγ if a source of B7 is present, such as would be provided by L cells that express high levels of B7 as a result of transfection. It was also shown that other agents that inhibit macrophage functions such as interleukin-4 and TGFβ fail to block this co-stimulatory activity and fail to inhibit the induction of B7. This understanding of the regulatory activity of IL-10 promises to provide new insights into ways to enhance or suppress immune responses (L. Ding, L.-Y. Huang, R.N. Germain and E.M. Shevach, LI/NIAID; P. Linsley, Bristol-Myers Squibb).

Development of Lymphokine-Producing Phenotype of Naive CD4 T Cells is Largely Determined by IL-4 and IL-12

Naive CD4+ T cells produce IL-2 but little IL-4 or IFNγ. Upon priming, these cells differentiate into cells that dominantly produce either IFNγ (TH1-like cells) or IL-4 (TH2-like cells). To understand what factors determine whether the dominant lymphokine produced will be IFNγ or IL-4, LI scientists have utilized T cells derived from mice transgenic for genes specifying a T cell receptor specific for a cytochrome C peptide in association with the class II MHC molecule I-E^k. This allowed an examination of in vitro priming to an authentic antigen. It was observed that priming for the production of IL-4 required the presence of IL-4 itself during the priming process. IFNγ diminished such priming, but only when suboptimal amounts of IL-4 were present. Different types of antigen-presenting cells (e.g. dendritic cells, activated B cells, I-E^k-transfected fibroblasts) differed in their potency but were not deterministic of the priming process. In every case, priming in the presence of IL-4 gave rise to IL-4-producing cells and priming in the presence of anti-IL-4 failed to do so. Priming for IFNγ-producing cells could be obtained in the absence of IL-4. IL-4 strikingly inhibited such priming. A substantial (~9-fold) enhancement in priming for IFNγ production was obtained if IL-12 was added to the cultures. In this case, IL-4's inhibition for priming for IFNγ production was markedly lessened. Thus, in the presence of IL-12, IL-4 diminished priming for IFNγ production by ~50% while in the absence of IL-12, IL-4 cause a 95% inhibition in priming for IFNγ production. The effect of IL-12 in priming for IFNγ-production appears to be mediated by direct action on the T cells and does not depend upon the presence of IFNγ during the priming process. These results should allow the development of strategies to direct primary immune responses either to dominant IFNγ production with striking cellular immunity and substantial effectiveness against intracellular microorganisms or to dominant IL-4 production, with good induction of antibody and diminished immunologically-mediated inflammatory responses (R.A. Seder and W.E. Paul, LI/NIAID; R. Gazzinelli and A. Sher, LPD).

IL-4-Producing T Cells Can be Anergized by Superantigens and Can be Reactivated by Infection with Helminths

Over the last several years, it has become clear that long term clones of IL-2-producing T cells could enter a state of *anergy* , in which they lost the capacity to produce IL-2 in response to antigenic stimulation but were not eliminated. A similar state of anergy can be induced in vivo in T cells responding to activation with superantigens, such as staphyloccal enterotoxin A (SEA). It had been postulated that this anergic state would be limited to the set of T cells that produce IL-2 and IFNγ but that those cells producing IL-4 would not be subject to such anergy. LI scientists have demonstrated that SEA treatment does induce a striking state of T cell anergy in IL-4-producing T cells. Thus, if mice are treated with IL-4 and SEA, a state of immunity is induced such that in vitro stimulation of these cells with SEA elicits production of IL-4. However, if the mice receive a second injection with SEA six days after the initial immunization, their capacity to produce IL-4 in response to in vitro challenge is completely inhibited although the T cells that are specific for SEA are not eliminated. This indicates that cells that have already made a commitment to the production of IL-4 are capable of being silenced or anergized by SEA, just as are cells capable of producing IL-2. However, infection of these anergized mice with the helminthic parasite, *Nippostrongylus brasiliensis*, which normally causes an immune response marked by vigorous IL-4 production, results in the reactivation of the anergized cells. These cells now produce substantial amounts of IL-4 upon re-challenge in vitro with SEA. The demonstration that IL-4-committed T cells can be anergized indicates that such T cells, which are important inducers of allergic responses, can be silenced even after they have been primed. Further theses results also indicate that powerful stimulation, as can be provided by particular infectious agents, can reverse the state of anergy and thus could, under special circumstances, induce a state of autoimmunity (M. Rocken and E. M. Shevach, LI/NIAID; J.F. Urban, USDA).

Apoptotic Cell Death in T Cells Stimulated With IL-2 Requires Entry into the S Phase of the Cell Cycle

Stimulation of resting T cells with antigen or anti-receptor antibodies leads to cell activation and proliferation. By contrast, stimulation of T cells that have recently been induced to enter the cell cycle by IL-2 results in cell death through apoptosis. Indeed, it appears that treatment of mice with IL-2 in the course of intense antigenic stimulation can result in clonal elimination through receptor-stimulated apoptosis. This process has been termed *propriocidal regulation*. LI scientists have extended their understanding of this process in two important ways during the past year. They have shown that the induction of apoptotoic sensitivity to receptor-mediated stimulation is not limited to the action of IL-2. IL-4 will also have such an effect in cells that are responsive to IL-4. This suggests that the effect of IL-2 may not be due to the activation of an *IL-2-specific* apoptosis-inducing gene but possibly may be reflected in its stimulation of the cell to enter and progress through the cell cycle. To examine this possibility, the effect of various cell cycle inhibitors to block apoptotic death was examined. It was observed that aphidicolin, an inhibitor that blocks cells near the G1/S boundary, did not prevent apoptosis in cells treated with IL-2 prior to receptor-mediated stimulation. By contrast, inhibitors that acted earlier in G1, such as deferoxamine and mimosine, exerted a striking protective effect. These results suggest that some critical event in G1 or early S phase, between the points at which deferoxamine and mimosine, on the one hand, and aphidocolin, on the other hand, block progress through the cycle is essential for the induction of apoptotic death. These results also provide one of the first group of pharmacologic agents that block apoptosis (S. Boehme and M.J. Lenardo, LI/NIAID).

Poly-ADP-Ribose Transferase Plays an Important Role in Cell Death Mediated by Cytotoxic Lymphocytes

Cytotoxic T lymphocytes cause the death of their target cells through processes that include DNA damage. LI scientists have been interested in the possibility that this target cell DNA damage may contribute to cell death through indirect means. During the past year, they have tested the hypothesis that the activation of poly-ADP-ribose transferase (pADP-RT) by DNA strand breaks may be an important step in the lytic process, possibly be depleting cellular NAD and ATP. Inhibitors of pADP-RT, such as 3-aminobenzamide, benzamide and nicotinamide, inhibited CTL-mediated lysis. The effect of the inhibitors was not due to blocking of lethal hit delivery since the DNA fragmentation that results from interaction of CTL with target cells was not blocked. Similarly, the inhibitors did not block CTL-mediated lysis of sheep erythrocytes, a non-nucleated target cell. A second important line of evidence implicating pADP-RT in the lytic process came from the use of a pADP-RT-deficient cell line. This line displayed relative resistance to CTL-induced lysis, although it displayed normal sensitivity to CTL-mediated DNA fragmentation and to antibody and complement mediated cell lysis. These results lead to the conclusion that the activation of pADP-RT can contribute to the CTL-induced lysis of some target cells. However, the data also indicate that other target cells, such as sheep erythrocytes, can be efficiently lysed without involvement of pADP-RT. Thus it appears that multiple molecular pathways are involved in target cell death in response to CTL. The relative contribution of pADP-RT activation will depend upon the action of these distinct pathways in target cells of different type (F. Redegeld and Sitkovsky, LI/NIAID).

Honors, Awards and Scientific Recognition

Laboratory of Immunology scientists play important roles in the U.S. and international scientific communities. They serve on editorial boards of many scholarly publications, on the advisory boards of major organizations, and act as organizers and major participants in international and national scientific meetings.

LI scientists have major editorial responsibilities for scientific journals and publications. Dr. Ethan Shevach is a member of the Council of Biology Editors. He serves on the editorial boards of the *Journal of Immunological Methods*, *Cellular Immunology*, and the *Journal of Biomedical Science*. He is section editor for immunology of *Life Sciences* and an editor of *Current Protocols in Immunology*.

Dr. William Paul is the editor of the *Annual Review of Immunology* and of the advanced textbook *Fundamental Immunology*, the third edition of which is now in press. He is an advisory editor of the *Journal of Experimental Medicine*, an associate editor of *Cell*, a contributing editor of the *Proceedings of the National Academy of Sciences* and a member of the editorial boards of *Immunological Reviews* and *Cytokine*. He is also a transmitting editor of *International Immunology* and a corresponding editor of the *Proceedings of the Royal Society, Series B*.

Dr. Ronald Germain is an advisory editor of the *Journal of Experimental Medicine*. Dr. Rose Mage is a member of the editorial board of *Immunogenetics*. Dr. David Margulies is an editor of *Current Protocols in Immunology* and an associate editor of the *Journal of Immunology*. Dr. Michail Sitkovsky is an associate editor of the *Journal of Immunology* and co-editor of *Cytotoxic Cells: Generation, Recognition, Effector Functions, Methods*, published by Birkhauser, New York. Dr. Lenardo is a member of the editorial board of *Molecular and Cellular Biology*. Dr. John Inman is a member of the editorial board of *Analytical Biochemistry* and is an advisory editor of *Molecular Immunology*.

Dr. Paul is a member of the Medical Advisory Board of the Howard Hughes Medical Institute; of the Committee to Visit the Division of Medical Sciences of the Board of Overseers of Harvard College; of the Fellowship Committee of the Cancer Research Institute; of the External Advisory Committee of the Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto; and of the Advisory Committee of the Department of Molecular Biology, Princeton University. He completed a term as a member of the Board of Scientific Consultants of the Memorial-Sloan Kettering Cancer Center.

Dr. Paul is Secretary-General of the Ninth International Congress of Immunology, to be held in San Francisco in 1995 and is chairman of the Congress' Executive Committee. He is a member of the board of the Mid-Winter Conference of Immunologists.

Dr. Paul was elected to fellowship in the American Academy of Arts and Sciences. He received the sixth annual Duke University Award for Excellence in Immunologic Research. During the past year, he presented the Annual Lecture at the Dutch Society of Immunology Meeting in Amsterdam and the Henry G. Kunkel Memorial Lecture at the Johns Hopkins University School of Medicine. He was the first annual Baruj Benacerraf Lecturer in Immunology at Harvard Medical School. He was a Wellcome Visiting Professor at the State University of New York at Stony Brook and Distinguished Professor of Immunology for 1993 at the University of California, Irvine.

Dr. Paul was an invited lecturer at the conference on Cytokines and Cytokine Receptors in Health and Disease, sponsored by NHLBI, at the William B. Coley Symposium on Frontiers of Immunology and Cancer Immunology, at the combined Keystone Symposia on B Cell Immunobiology and on Molecular Aspects of B Lymphocyte Differentiation, in Taos NM, at the international workshop on Combined Vaccines and Simultaneous Administration, sponsored by CBER, FDA; and at the conference on T Cells and Cytokines in Health and Disease, held at Trinity College, Oxford.

Dr. Shevach received the Distinguished Service Medal of the United States Public Health Service, the Distinguished Alumnus Award of the Boston University School of Medicine Alumni Association, and the Distinguished Service Award of the American Association of Immunologists. He is a member of the Scientific Advisory Board of the American Leprosy Foundation.

Dr. Germain is membership chairman of the American Association of Immunologists. He is a member of the Scientific Advisory Board, Ruggiero Ceppellini Advanced School of Immunology, Naples, Italy. He servers as Director of the NIAID Grand Rounds Program.

Dr. Germain was an invited lecturer at the Fundacion Juan March Symposium on the Structure of the Major Histocompatibility Complex, held in Madrid, at the MD Anderson annual symposium entitled Immunology and Cancer: Cellular and Molecular Mechanisms, held in Houston; at the Children's Vaccine Initiative Symposium in Bethesda; at the annual meeting of the British Society for Immunology; at the NIH Clinical Center Grand Rounds; at the New York Academy of Sciences symposium on Immunotherapy of Cancer with Vaccines, held in Washington, DC; at the Keystone Symposium on Emerging Principles of Vaccine Development: Antigen Processing and Presentation, in Taos NM; at the annual meeting of the Swiss Society for Immunology, in Geneva; at the international workshop on Combined Vaccines and Simultaneous Administration, sponsored by CBER, FDA; and at the Roche Milano Ricerche symposium Towards Selective Immunotherapy of Autoimmune Diseases, in Milan.

Dr. Germain was the Karl Popper Lecturer at the University of Nebraska and a plenary lecturer at the Annual Meeting of the American Society for Clinical Investigation.

Dr. Margulies was an invited lecturer at the symposium in honor of Dr. Matthew Scharf, "Immunology Today", at the Albert Einstein College of Medicine and was co-chairman of a minisymposium at the NIH Research Festival, Antigen Processing and Presentation.

Dr. Lenardo received the NIH Director's Award. He was an invited lecturer at the New England Immunology Conference; at the Gordon Research Conference on Immunology and Immunochemistry; at the FASEB Conference on Lymphocytes and Antibodies; and at the FASEB Conference on Autoimmunity.

Dr. Sitkovsky was chairman of a session at the Fifth International Cytotoxicity Workshop, in Israel and co-chairman of a workshop on Mechanisms of Cell-Mediated Cytotoxicity at the NIH Research Festival.

Members of the Laboratory of Immunology have presented research seminars at universities, medical schools and research institutes in the United States and abroad.

The Laboratory of Immunology was particularly gratified to learn that one of its former post-doctoral fellows, Professor Mark Davis of Stanford University School of Medicine, had been elected to the National Academy of Sciences USA.

Administrative, Organizational and Other Changes

The Laboratory of Immunology is a major training center for young immunologists. During the past year, several scientists completed post-doctoral training or sabbatical periods in the Laboratory. Among these are Jean-Louis Boulay, Mary-Pat Corr, Elizabeth Bonney, Melissa Gregory, Rolf Koening, Frank Redegeld, Randall Ribaudo, Martin Rocken, Dan Salomon and Robert Seder. Each of these individuals made important contributions to the Laboratory of Immunology research program. It is anticipated that they will have very productive research careers and that many will attain leadership positions in modern immunology or related fields.

During the past year, several scientists joined the laboratory of immunology as post-doctoral fellows. They include Sylvain Fleury, Marie Jelonek, Masahiro Koshiba, Joaquin Madrenas, Christopher Mojcik, Keats Nelms, Robert Ortmann, Richard Pospisil, Caetano Reis e Sousa, Shozo Sakuma, Benjamin Segal, Vladimir Sverdlov, Li Yin and Tomohiro Yoshimoto. It is expected that they will continue the tradition of excellence established by a long series of trainees in the Laboratory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 0030-25 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Paul	Chief	LI/NIAID
Others:	Jeffrey Thomas	Guest Researcher	LI/NIAID
	Charles Chu	Staff Fellow	LI/NIAID
	John Ryan	IRTA	LI/NIAID

COOPERATING UNITS *(if any)*
CBER, FDA (E. Max); Molecular Allergy and Immunology Section, NIAID (J.-P. Kinet); Roswell Park Memorial Institute (V. Chapman); Karolinska Institute (C. I. E. Smith and I. Vorechovsky); University of Umea (P. Sideras)

LAB/BRANCH
Laboratory of Immunology

SECTION
General Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The X-linked immunodeficiency of CBA/N mice is caused by a gene on the X-chromosome designated *xid.* This defect is characterized by unresponsiveness to polysaccharide antigen, low serum IgM and IgG3 levels and diminished numbers of B cells. In order to positionally clone this gene, an F1 between CBA/N and Mus musculus Skive mice was made. The F1 females were crossed to male CBA/N mice. A backcross of over 1100 animals was typed for *xid* phenotype and for a variety of genetic markers. This led to the precise mapping of *xid* on the mouse X-chromosome. Indeed, a simple sequence repeat marker, *DXMit3*, failed to recombine with *xid* in any of the members of the backcross. A set of YACs was obtained that expressed DXMit 3. The fine mapping of *xid* indicated that it was in a region of the mouse X chromosome homologous to the region in which the gene determining human X-linked agammaglobulinemia (Xla) was found. This disease is cause by mutations in the kinase domain of the Bruton's agammaglobulinemia tyrosine kinase (*btk*). Using *btk* specific primers, it was shown that *DXMit 3* positive YACs all bore *btk*. Moreover, based on a polymorphism in *btk*, it was shown that no recombinations existed between *btk* and *xid*. Sequencing of *btk* of CBA/N mice and of their nearest relative, CBA/CaHN, revealed a point mutation that converted position 28, within the unique domain of the kinase, from arginine to cysteine. In a total of 7 mammalian species, position 28 of *btk* is always arginine, arguing that this point mutation is very likely the cause of the *xid*-determined immune defect.

Studies of Ig class switching have been carried out with a quantitative and specific assay, digestion-circularization PCR (DC-PCR), which allows one to measure the progress of DNA deletion in the course of the switching process. In B cells stimulated with lipopolysacchartide (LPS) and IL-4, a high degree of switching to IgG1 occurs. DC-PCR assay reveals that there is sufficient DNA deletion occurring in the course of in vitro switching to account for all switched cells. This indicates that, if other mechanisms play a role in switching, they do so mainly as a prelude to deletional switching.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00035-18 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Design and Synthesis of Immunomodulators, Immunogens, and Vaccine Constructs

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	John K. Inman	Section Chief	LI, NIAID
Others:	Andrew Lees	NIH Special Volunteer	Dept of Medicine, USUHS
	Patricia F. Highet	Technician	LI, NIAID

COOPERATING UNITS (*if any*) Dept Medicine, USUHS (F.D. Finkelman, J.J. Mond); New York Univ Sch Medicine (P.K.A. Mongini); Food & Drug Admin (B. Golding, H. Golding); State Univ., Utecht , The Netherlands (H. Snippe); Pathology Lab., NCI, NIH (H.C.Krutzsch, D.D. Roberts); Dept. of Microbiology and Immunology, Virginia Commonwealth University (K.L. McCoy).

LAB/BRANCH
Laboratory of Immunology

SECTION
Bioorganic Chemistry Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	(1.0 not paid through NIH) 1.4	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project is directed toward the design and synthesis of immunogens and immunmodulators for collaborative studies in basic and applied immunology. Much of this work has been focused upon (1) studying mechanisms of activation of B cells through their specific antigen-binding receptors (membrane immunoglobulins), (2) developing novel and highly effective ways to stimulate specific humoral and cellular immune responses, and (3) translating these findings into potentially practical approaches to the immunization of humans against infectious diseases (e.g., malaria, AIDS, diphtheria-pertussis-tetanus, and H. influenzae Type b infections). Emphasis has been placed on studying the effects of polyvalent presentation of biologically active molecules (antibodies, peptides, polysaccharides, ligands, haptens, etc.), covalently linked to soluble high molecular weight polymers, on cell cultures or experimental animals. Soluble conjugates permit study of dose-response relationships and the quantitation of signal amplification due to multivalence by direct comparison with unconjugated effector molecules. An example has been collaborative studies on B cell activation by means of anti-immunoglobulins multiply coupled to high molecular weight dextran to serve as panclonal surrogates for hapten-specific type-2 thymus independent immunogens. New reagents and methods have been developed in this Section for synthesizing conjugates used for investigating novel approaches to synthetic vaccines. Enhanced humoral immune responses have been observed to heterodimers of antigen and anti-membrane proteins and to polyvalent conjugates of antigens with soluble, high molecular weight polymers (e.g., dextran). Rapid, large, specific antibody responses have been elicited with these constructs which were effective without adjuvant. Conjugates based on lipopolysaccharide from B. abortusas a carrier and adjuvant are being actively investigated as potential therapeutic vaccines for treating AIDS. Collaborative studies have been initiated on the biological responses, *in vitro* and *in vivo*, to synthetic peptides linked to polymer carriers. The sequences are taken from the large, adhesive glycoprotein, thrombospondin, that interacts with cells and matrix structures, serving as both a positive and negative modulator of motility, adhesion and proliferation. The goal is to develop selective biological response modifiers for controlling tumor metastases and various lymphocyte

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00036-28 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	M. Fitts	IRTA Fellow	LI, NIAID
	H.-T. Chen	Visiting Scientist	LI, NIAID
	P.D. Weinstein	IRTA Fellow	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID
	R. Pospisil	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(if any)* DAD, USAMRID, Ft. Detrick, Frederick, MD (A. O. Anderson); Dept Biol Sci Florida State U., Tallahassee, FL. (K.Roux); Dept Pathol/Lab Med Univ. Florida Coll. Med., Gainesville, FL. (W. T. McCormack); Dept Pathol Ohio State Univ., Columbus, OH (N. Harindranath); Dept Tumor Cell Biol St. Jude Children's Res. Hosp., Memphis TN (L. M. Hendershot); Dept Microbiol., Med. Coll. Ohio, Toledo, OH (D. W. Metzger); Spring Valley Labs. Inc. (R. Shaw)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We use techniques of immunogenetics and of molecular biology to study the rabbit immunoglobulins (Igs), and related genes such as the recombination activating genes RAG-1 and RAG-2 which are necessary for gene rearrangements to occur during lymphocyte development. We investigate the development of anatomical sites such as appendix follicles and germinal centers in gut associated lymphoid tissues and the regulated expression and sequence diversification of Ig genes during lymphoid cell development. Membrane IgD has not been definitively identified on rabbit B cells; the genetic region which should contain the δ gene has been cloned, but sequences corresponding to δ heavy chain exons have not been identified. We have identified two surface immunoglobulin complexes on rabbit peripheral blood B cells that possess heavy chains of similar apparent molecular size confirming previous reports of the presence of a second surface receptor expressed on rabbit B cells which may be IgD or an IgM-related molecule. In noncovalent association with one or both of these receptors are several proteins that are components of surface immunoglobulin (mIg) complexes expressed on rabbit B cells. Three proteins (42 kDa, 37kDa, 36kDa) were found in association with IgM expressed on B cells from peripheral blood and spleen. These proteins may be the rabbit homologues of murine Ig-β (B29), Ig-γ (truncated B29) and Ig-α (mb-1). Lectins are allowing us to dissect rabbit appendix follicles and germinal centers into subcompartments based on local expression of glycosylated cell surface receptors. We found two additional IgM-associated protein complexes containing 36kDa and 34kDa proteins in lysates from individual follicles isolated from the appendix. The appendix may be functioning early in development like the chicken bursa or sheep ileal Peyer's patch. However, the rabbit appendix does not involute but may alter its function and become a secondary lymphoid organ in the adult.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00134-31 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard Asofsky	Section Chief	LI, NIAID
Others:	J. Little	Staff Fellow	LI, NIAID
	F. Zhao	Visiting Associate	LI, NIAID (6 months)
	H.-K. Chu	Visiting Associate	LI, NIAID (6 months)
	N. Ohori	Visiting Fellow	LI, NIAID
	A. Brooks	Biological Technician	LI, NIAID.

COOPERATING UNITS (if any)

DEB, NICHD (M. Flack); DCBD, NCI (H. Coon)

LAB/BRANCH

Laboratory of Immunology

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TH 2.2 is a cloned hybrid cell line (Lymphoma X B-lymphocyte) which is induced by bacterial lipopolysaccharide (LPS) to a reduced rate of growth and to the secretion of cytokines, including IL-3, IL-6 and GM-CSF. Individual clones obtained in the presence of LPS are very heterogeneous with respect to size, and to the number and amounts of products secreted. Almost all clones return to the parental phenotype. We studied the effects of treatment with LPS and with cytokines on the behavior of TH2.2 during induction. Pretreatment of cells with LPS prior to induction produced profound unresponsiveness to LPS: Inhibition of growth and induction of cytokine secretion was blocked. This state of "tolerance" was very long lasting, even after treated cells were permitted to grow in regular growth medium in the absence of LPS. Secretion of GM-CSF was still very suppressed after 28 passage generations; secretion of IL-3, IL-6 had recovered by this time, although it had been suppressed in passages 1-17. Cells were still partly resistant to the growth-inhibiting effects of LPS at passage 28. Thus, treatment with LPS produces unresponsiveness to its own action, as measured by four functional parameters, and each of the parameters recovers at a different rate. Pretreatment of TH 2.2 with TNF-α, IFN-γ, IL-1, and IL-4 could effect the magnitude of the response to LPS by enhancing the secretion of GM-CSF and/or reducing the secretion of IL-3 to subsequent challenge with LPS.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI00224-12 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptors, Co-Receptors, and Counter-Receptors for T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.M. Shevach	Section Head	LI, NIAID
Others:	L. Ding	Visiting Fellow	LI, NIAID
	M. Rocken	Visiting Associate	LI, NIAID
	V. Rostapshov	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*

Department of Physiology, Wayne State University School of Medicine (Dr. R. Gala)

LAB/BRANCH
Laboratory of Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The interaction of the T cell receptor (TCR) with its specific ligand, peptide-MHC, is only one component of the events required for the activation of antigen-specific T cells. The primary goals of our studies are to characterize cell surface antigens (co-receptors) and their soluble or cell associated ligands (counter-receptors) which play critical roles in cell-cell interaction and in the interaction of T cells with their environment. We have concentrated out efforts in a number of distinct areas: 1) We have shown that the cytokine, interleukin-10 (IL-10), inhibits T cell activation by acting on macrophages and not other types of accessory cells. We have further elucidated the mechanism of action of IL-10 on the inhibition of macrophage costimulatory activity and shown that IL-10 selectively inhibits the upregulation of expression of the B7 antigen which is the cellular counter-receptor for the T cell antigen CD28. The regulation of B7 expression by IL-10 may determine whether T cells become primed or are rendered non-responsive during the generation of an immune response. 2) One model of immunologic tolerance involves the induction peripheral T cell anergy by the injection of adult mice with Staphylococcal enterotoxins. To further characterize the state of a tolerant T cell in vivo, we have established an in vivo model that generates enterotoxin specific IL-4 production. This IL-4 pathway was highly susceptible to tolerogenic signals in both naive T cells and in T cells primed for IL-4 production. The tolerized IL-4 pathway could only be reactivated by expansion during parasite mediated polyclonal activation. These studies should facilitate our understanding of the pathogenesis of autoimmune disease following infectious disease. 3) A number of studies have suggested that the pituitary hormone, prolactin (PRL), plays an important role in immunoregulation. We have demonstrated that T cells, B cells, and macrophages express PRL-receptors and that expression of this receptor on T cells is upregulated during the course of T cell activation. No role for maternal PRL could be defined in the development of the immune system, as the thymic abnormalities seen in the offspring of mothers treated with the PRL secretion inhibitor, bromcriptine, did not appear to be secondary to the decreased level of PRL in their offspring. However, T cells from dwarf mice which have a genetic defect in the synthesis of PRL as well as other pituitary hormones were shown to have a defect in their ability to upregulate the expression of the IL-2 receptor following T cell activation in vivo and this defect could be overcome by administration of PRL.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00226-12 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	Others: M. Fitts	IRTA Fellow	LI, NIAID
	H.-T. Chen	Visiting Scientist	LI, NIAID
	P.D. Weinstein	IRTA Fellow	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID
	R. Pospisil	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(if any)* DAD, USAMRID, Ft. Detrick, Frederick, MD (A. O. Anderson); Dept Biol Sci Florida State U., Tallahassee, FL (K. Roux); Dept Pathol/Lab. Med. Univ. Florida Coll. Med., Gainesville, FL. (W. T. McCormack); Dept Pathol Ohio State Univ., Columbus, OH (N. Harindranath); Dept Tumor Cell Biol. St. Jude Children's Res. Hosp., Memphis TN (L. M. Hendershot); Dept Microbiol, Med. Coll. Ohio, Toledo, OH (D. W. Metzger); Spring Valley Labs. Inc. (R. Shaw)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We study genes of the rabbit immune system by techniques of molecular biology and immunology. Alternative mechanisms such as gene conversion (g.c.) appear to contribute to the diversification of the antibody repertoire in the rabbit. The rabbit appendix develops rapidly between birth and 6 weeks of age, when it seems to reach its maximum size. We analyzed diversification from germline VH gene sequence in individual rabbit appendix follicles. Our results suggest that g.c. is occurring to diversify germline gene sequences in the dark zone cells of the appendix follicles of six week old rabbits. Some changes may have been due to either g.c or somatic hypermutation. Earlier, we found that a relatively small deletion of a segment containing 3' VH genes leads to a mutant phenotype in *Alicia* (*ali*) rabbits. We found that the products of the first functional VH gene (VH4a2) or VH4a2-like gene(s) were expressed in two to eight-week-old mutant *Alicia*; expression of a-negative genes was also elevated but the relative proportions of a-negative decreased as the relative levels of expression of the VH4a2 or VH4a2-like gene(s) increased. The appearance of sequences resembling that of the VH1a2 gene which is deleted in the mutants may be due to g. c. that altered the sequence of the rearranged VH4a2; rearrangement of upstream VH1a2-like genes may also occur later in development. We described the rabbit RAG locus and genomic and expressed RAG-1 and RAG-2 genes. Genetic polymorphism of the rabbit RAG-2 gene was observed by sequencing and Southern analyses. Expressed RAG-2 cDNAs with different sequences were both functional in a recombinase assay. RAG-1 and RAG-2 positive cells can be found in the 6 week normal rabbit appendix. Scattered throughout the M cells pockets, the corona, and both the dark and light zones of germinal centers. PCR allows us to specifically identify excision circles resulting from VL to JL DNA rearrangements. Circular products reflecting VLJL rearrangements were detected in the appendix of two week-old and adult animals.

U S GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00349-10 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	R. Koenig	Visiting Associate	LI, NIAID
	S. Sadegh-Nasseri	Special Volunteer	LI, NIAID
	P. Romagnoli	Visiting Associate	LI, NIAID
	S. Fleury	Visiting Fellow	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	A. Rinker, Jr.	Research Technician	LI, NIAID
	L-Y. Huang	Research Technician	LI, NIAID

COOPERATING UNITS *(If any)*

E. Bikoff, E. Robertson; L. Stern; D. Wiley (Harvard University)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	2.6	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Class II MHC (Ia) gene products play critical roles in a variety of T and B lymphocyte responses. Biochemical and functional analyses have been used to investigate the relationship between class II structure and peptide antigen presentation. Class II molecules were found to undergo a characteristic structural change upon binding of appropriate peptides. The kinetics of class II peptide binding have been re-examined and two distinct phases of binding identified; a rapid initial binding with a rapid off rate, and a slow accumulation of long-lived binary complexes which is markedly augmented at the pH of the class II (endosomal) processing compartment. Studies in invariant chain-deficient mice have shown the critical role of this non-polymorphic protein in regulating class II assembly and folding within cells, as well as the capture of peptides derived from exogenous antigens. These new insights into the biochemical behavior of class II provide new understanding of how antigen capture by class II molecules occurs within living cells.

In addition to binding peptide and being recognized by the clonally distributed T cell receptors, class II molecules participate in T cell selection in the thymus and activation in the periphery by interacting with the CD4 molecule that is also the receptor for HIV-1. We have used site-directed mutagenesis to define the site(s) of interaction of class II molecules with CD4, and demonstrated that a short continuous region in the β2 domain of the class II molecules plays a major role in this interaction. We have also tentatively mapped a second region affecting CD4-class II interaction to the class II α chain. This latter observation may relate to the discovery that class II molecules form dimers of dimers when crystallized, and provides a new paradigm for assembly of the minimal T cell receptor signaling complex. Together with the studies on class II folding, transport, and control of peptide binding by the polymorphic domain, these experiments will provide new insight into the molecular mechanisms involved in antigen recognition by, and activation of T lymphocytes.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00394-10 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Charles Hoes	Biologist	LI, NIAID
	Maripat Corr	Medical Staff Fellow	LI, NIAID
	Rosemarie Hunziker	Sr. Staff Fellow	LI, NIAID
	Steven Kozlowski	Medical Staff Fellow	LI, NIAID

COOPERATING UNITS *(if any)*
MB, NCI (T. Takeshita, J. Berzofsky); LB, NCI (M. Mage, L. Lee); F. Karlhofer and W. Yokoyama, Mt. Sinai School of Medicine; A. Bothwell (Yale University)

LAB/BRANCH
Laboratory of Immunology

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of these studies has been on the functional role of the MHC class I molecule and the development of model systems for examining this function in vitro. Specific accomplishments during the past year include: 1) the characterization of proteolytic pathways involved in the extracellular processing of different class I restricted peptides; 2) the characterization of the minimal peptides derived from the HIV-1 gp 160 envelope glycoprotein that are presented by both H-2D^d and I-A^d; 3) the biochemical and functional analysis of a set of single chain class I molecules generated by engineering techniques; 4) the evaluation of the role of the MHC class I molecule H-2D^d in the signalling of a subset of NK cells bearing the cell surface marker Ly-49; 5) the evaluation of T cell receptor/class I interactions.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00403-10 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	L. Racioppi	Visiting Associate	LI, NIAID
	J. van Meerwijk	Visiting Fellow	LI, NIAID
	M. Gregory	Special Volunteer	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	J. Madrenas	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*

LMI, BRMB, DCT, NCI (L. Matis); Basel Institute for Immunology (F. Ronchese)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.7	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antigen-specific T lymphocyte activation occurs through the clonally distributed T cell receptor (TCR). The available set of αβ TCR is dictated by both positive and negative intrathymic selection events. The molecular basis of this selection, in particular, how bias for self-MHC presented peptides is achieved, is unknown. Furthermore, the quantitative and qualitative relationships between receptor occupancy and signaling for differentiation, whether in the thymus or the periphery, are also poorly understood. This project uses cellular and molecular tools to study the development of the T cell repertoire and the activation of T cells upon ligand engagement.

Analysis of thymocyte subsets in normal and MHC class I-deficient mice allowed the identification of a novel thymocyte subpopulation (CD4 intermediate CD8hi TCRhi) that is in the process of becoming a CD8+ mature T cell following receptor recognition of MHC class I ligand. These studies unexpectedly revealed that some cells initiate development in the CD8 lineage by recognition of MHC class II molecules. This finding casts doubts on a model of T cell differentiation that ascribes initial lineage commitment to a match between the binding specificity of the CD4 or CD8 co-receptor and the MHC class of TCR ligand.

We have found that some TCR ligands evoke only a subset of T cell effector responses, and that such variant ligands can antagonize cytokine production induced by known receptor agonists. These findings may provide a new approach to the control of autoimmune diseases and a means of controlling the type of effector responses elicited by component vaccine formulations. Finally, because IL-2 (T cell growth factor) production by $CD4^+$ T cells is known to be very dependent on co-stimulatory signals distinct from those arising from occupancy of the clonally distributed αβ T cell receptor, we have begun exploring the pathways that lead to expression of the major co-stimulatory ligand(B7) on B cells. Our work demonstrates a critical role for membrane-mediated T cell: B cell signaling involving the interaction of T cell-expressed CD40 ligand and B cell expressed CD40, and suggests that expression of CD40 ligand (the locus of the defect in the hyper IgM immunodeficiency syndrome) may require co-stimulatory signals.

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00425-09 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lymphocyte Physiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. M. Chused	Senior Investigator	LI, NIAID
Others:	Shigeru Ohno	Visiting Fellow	LI, NIAID
	P. E. Hart	Biologist	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Transmembrane Signalling Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized. These studies have revealed extensive"feed forward" and "feed back" regulatory relationships between ion channel opening, membrane potential, activity of the calcium pump, and rate of phosphatidyl inositol turnover. These mechanisms differ in the T, B and monocyte/granulocyte lineages. The process of signal transduction in thymocyte differentiation is being examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00427-09 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	S. Apasov	Fogarty Fellow	LI, NIAID
	F. Redegeld	Fogarty Fellow	LI, NIAID
	P. Smith	Research Associate	LI, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH
Laboratory of Immunology

SECTION
Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The understanding of the anti-viral and anti-tumor immune responses requires the studies of the molecular mechanisms of the lysis of virus-infected or tumor cells by effector lymphocyte subpopulations. Thus, our efforts are directed toward the: A) implication of specific CD8+ and/or CD4+ cells in the anti-tumor response and B) identification of key enzymes in CTL and in target cells that participate in the "lethal hit delivery" and "reception".

A) With the recent development of mice with genetically disrupted β2-microglobulin (β_2m-/- mice) and with the deficiency of MHC class I expression the data were accumulated which indicated the low functional significance of the CD8+ CTL in anti-virus and transplantation immunity. We found that in contrast to published reports, the intraperitoneal immunization of β2-m -/- mice with alive tumor cells results in the accumulation of highly lytic and MHC class I specific CD8+CTL among peritoneal exudate lymphocytes (PEL) and in peripheral lymph organs. These CD8+CTL are similar to PEL CTL of normal mice in being responsible for tumor rejection except for the delay of tumor rejection in primary immune response and dramatic reduction of the Vβ5 and Vβ6 TCR phenotype usage. It is concluded that CD8+ CTL are major contributors in the anti-tumor immunity and that peritoneaum provides a very favorable environment for their development. These results emphasize the advantages of using CD8+ CTL in intraperitoneal adoptive immunotherapy.

B) Poly ADP-rybose polymerse (PDDPRT) partially contributes into the CTL induced target cell death. The understanding of the mechanism of the TC death is important and we demonstrated that this enzyme is a part of a "suicidal" pathway, where CTL-induced DNA breaks, activate PADPRT which in turn depletes cellular NAD, ATP causing cell death. These results point to the PADPRT as possible target for immunomodulation, since the enhancement of PADPRT activity may result in more efficient lysis of tumor- or virus-infected cells by CTL.

U S GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00493-07 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
B Cell Stimulatory Factor-1 (BSFs-1)/Interleukin-4 (IL-4)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Paul	Chief	LI/NIAID
Others:	Robert A. Seder	Medical Staff Fellow	LI/NIAID
	Achsah D. Keegan	Staff Fellow	LI/NIAID
	Jean-Louis Boulay	Visiting Fellow	LI/NIAID
	Keats Nelms	IRTA	LI/NIAID
	Tomohiro Yoshimoto	Visiting Fellow	LI/NIAID
	John Ryan	IRTA	LI/NIAID
	Michael J. Lenardo	Senior Staff Fellow	LI/NIAID

COOPERATING UNITS *(if any)*

National Cancer Institute (J. Pierce and L-M Wong); Hebrew University-Hadassah Medical School (S.Z. Ben-Sasson)

LAB/BRANCH
Laboratory of Immunology

SECTION
General Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	6.5	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Naive CD4+ T cells produce interleukin-2 (IL-2) upon stimulation by antigen and antigen-presenting cells but can be induced to differentiate into cells that principally produce interleukin-4 (IL-4) or interferon gamma (IFNγ). The determination of which lymphokine is dominantly produced has a major impact on the protective value of the immune response. Utilizing CD4+ T cells from T cell receptor transgenic mice, it was shown that IL-4 itself is the principal factor determining whether T cells develop into IL-4 producers and, in addition, strikingly suppresses priming for IFNγ production. In the absence of IL-4, priming leads to cells that produce moderate amounts of IFNγ. Addition of IL-12 to the priming culture upregulates priming for IFNγ production and allows such priming to occur even in the presence of IL-4. The molecular basis of such priming is unknown but it appears likely that it involves regulation of lymphokine transcription. A study of the IL-4 promoter revealed that, in transient transfection assays using a CAT reporter gene, the sequence from -87 to the transcription start site exerts fully inducible IL-4 promoter-mediated CAT transcription in EL-4 thymoma cells that have been stimulated with phorbol esters. Two regions that contain copies of a consensus sequence (CS1 and CS2 - ATTTTCCNNTG) have been identified. Deleting CS2 causes a modest diminution in CAT transcription while deleting CS1 completely inhibits transcriptional activity. PMA-inducible binding factors that interact with CS2- and CS1-containing oligonucleotides have been identified; these appear to be different from one another. Multimerization of the CS1-containing oligonucleotide drives CAT transcription from a minimal promoter in TH2 but not TH1 cells suggesting this region may be critical to these distinctive lymphokine-producing phenotypes of the two cells. IL-4 action is mediated by binding to a high affinity receptor. This binding results in the phosphorylation of a 170 kDa substrate, designated 4PS, an analog of the insulin receptor substrate-1 (IRS-1). Phosphorylation of 4PS/IRS-1 is lost in truncation mutants of the receptor that delete the most membrane proximal tyrosine. The sequence around that tyrosine is homologous to the sequence around a comparable tyrosine in the insulin and IGF-1 receptors; this motif has been termed the I4R motif. A fusion protein containing the I4R motif binds the substrate and the kinase that can catalyze the phosphorylation of IRS-1.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00520-06 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Analysis of T Lymphocyte Responses to HIV Proteins

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	R. Germain	Section Head	LI, NIAID
Others:	W-H. Boehncke	Guest Worker	LI, NIAID
	A. Fox	Research Technician	LI, NIAID

COOPERATING UNITS (*If any*)

MB, NCI (J. Berzofsky, T. Takeshita)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

To develop therapeutic and vaccine strategies to combat HIV-1, the cause of AIDS, it is essential to understand the cell-mediated immune response to this agent. To pursue this goal, murine model systems for immunization, isolation of antigen-specific T cells, and *in vitro* analysis of T cells specific for HIV-1 proteins have been devised. Structure-function studies on a class II-restricted determinant have revealed how a small subset of residues controls binding to MHC molecules, how additional residues regulate the epitopic structure necessary for T cell recognition, and how it may be possible to improve the potency of vaccine antigens by removal of interfering groups in the natural antigen. In particular, an HIV-1 gp160-derived peptide termed T1 that is recognized by both mouse and human T cells together with class II MHC molecules was analyzed. A small subset of residues was found to be sufficient for binding to class II molecules, although not adequate for T cell recognition. Removal of a glutamic acid residue resulted in a 100 fold increase in the antigenic potency of the peptide assayed with a specific T cell clone, and this altered peptide was at least 10 times more effective on a mass basis in inducing responses in mice. This increased potency resulted from an improvement in the MHC class II binding properties of the peptide. These results demonstrate the critical role played in antigen activity by structural features that inhibit rather than contribute to interaction with either MHC molecules or T cell receptors. Recognition of this effect will allow improvement of vaccine constituents and more effective identification of immunogenic regions of proteins when analysis for the absence of interfering groups is included with scans for motifs necessary for promoting MHC molecule binding.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00545-05 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	P.Romagnoli	Visiting Associate	LI, NIAID
	E.Bonney	Medical Staff Fellow	LI, NIAID
	F. Castellino	Visiting Associate	LI, NIAID
	L-Y. Huang	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

LVD, NIAID (J. Yewdell); Univ. of Oslo (O. Bakke)

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Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

αβ T cells respond to complexes of peptide antigen and MHC molecules. The way in which protein antigens are transformed into peptides suitable for such binding and the intracellular pathways followed by MHC molecules both before and after peptide association are critical to our understanding of T cell immunity. We have used normal and gene transfected cells to examine these issues. Although class II molecules can assemble in the absence of invariant chain, the presence of this latter protein markedly augments proper class II formation in the ER. Following movement through the Golgi, invariant chain provides targeting signals that determine the subsequent movement of newly synthesized class II molecules to endocytic compartments. Our studies in transfected COS cells as well as using a combination of pulse-chase immunoprecipitation and gradient density organelle fractionation of B lymphoblasts have shown that early endosomes are the initial site of entry into the endosomal pathway, but that removal of invariant chain and significant amounts of peptide loading occur during or upon movement to late endosomes. Invariant chain has the capacity to alter the structure of early endosomes and change the rate of movement of endocytosed proteins through this early sorting compartment. Langerhans cells play a central role in initial antigen presentation to naive T cells, and our studies suggest that these cells overexpress invariant chain during upregulation of class II synthesis during maturation, creating a change in endosomal flow akin to that observe in our transfected fibroblast model. These studies better define the late intracellular path of class II and begin to localize the specific site(s) of antigen loading in the class II pathway.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00565-04 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Family of κB Regulators for Genes in the Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	D. Jiang	Graduate Student	LI, NIAID
	M. Grilli	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
20	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Regulation of genes for several lymphokines as well as other molecules involved in the immune response depend on a 10 bp DNA sequence termed, κB. This sequence binds a growing family of nuclear proteins, several of which are related to the rel oncogene, that are capable of tightly governing transcription of these genes. Importantly, the κB sequence is found in the human immunodeficiency virus (HIV). A cardinal feature of the κB sequence is that it permits transcription in a highly regulated fashion both temporally and in appropriate cell-types for specific genes. We are attempting to elucidate how this specific regulation occurs. In studying the molecular activation of NF-κB in nontransformed T cells following stimulation by antigen and antigen-presenting cells (APCs), we found that exposure to APCs alone was sometime sufficient. NF-κB induction by APC's alone appeared due to the secretion of TNFα. These results thus establish an antigen receptor-independent pathway for the up-regulation of NF-κB during T cell activation.

We have also found that the microheterogeneity in DNA sequence among κB sites has regulatory significance. By studying normal non-transformed T lymphocyte clones we discovered a novel nuclear complex, termed NF-κC (previously called NF-CYT1) that interacts preferentially with a κB site in the interleukin-2 gene. The presence of NF-κC in a number of different biological conditions is inversely correlated with IL-2 gene expression in T cells. This suggests it may be a negative regulator. We have shown that NF-κC consists of a homodimeric complex of the NF-κB p50 subunit and that it can directly repress the function of the IL-2 promoter. Very significantly, this factor binds to the enhancer region of HIV. We postulate it may have a role in suppressing HIV viral transcription in resting T cells. Importantly, we have found that the binding activity of the NF-κC complex is governed by an inhibitory protein (IκC) that sequesters the NF-κC complex in the nucleus following antigen stimulation. Evidence currently suggests that the inhibitor of the p50 complex may be the proto-oncogene bcl-3, that is found at the breakpoint of chromosomal translocation in specific types of lymphomas.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00566-04 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Regulatory Events in Establishing Mature T Cell Tolerance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	Lixin Zheng	Visiting Fellow	LI, NIAID
	K. Bruhn	Research Technician	LI, NIAID
	J. Milner	Summer Student - MIT	LI, NIAID
	L. Chen	Summer Student - MIT	LI, NIAID

COOPERATING UNITS *(if any)*

LI, NIAID, Drs. William E. Paul and Keats Nelms

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

T cell tolerance has been found to occur in immature T cells in the thymus and also extrathymically in mature T cells. Our studies of mature T tolerance has revealed that they may undergo deletion or enter a functionally unresponsive state termed anergy. In anergy, a T-helper cell can be induced to "turn off" IL-2 production if it is stimulated through its T-cell receptor in the absence of costimulation. Previously we showed that regulatory proteins that govern IL-2 gene expression may be poorly activatable in response to antigen in anergic T cells. One important determinant of whether T cells will be normally activated by encountering an antigen/MHC complex is whether it is presented along with a co-stimulatory stimulus. Co-stimulation is the signal provided by the interaction of surface molecules such as CD28 (on the T cell) and B7 or BB-1 (on the antigen-presenting cell). The presence of co-stimulation seems to be required for the normal T cell proliferative response to antigen. To understand the role of co-stimulation in IL-2 production, we have studied the effects of co-stimulation on various elements in the IL-2 promoter. Thus far these studies indicate that the transcriptional function of certain cis elements in the promoter can be augmented by co-stimulatory influences. Further studies are in progress to further define the molecular mechanisms involved. Finally, an important aspect of the success of an immune response to an invading micro-organism appears to be the specialization of the helper T cell response towards cells that either produce IL-2 (T_H1 cells) or IL-4 (T_H2 cells). We have studied the regulatory elements in the promoter of the IL-4 gene and detected a transcriptional trans-activator and its binding site (CS-1) that are restricted to and crucial for expression in T_H2) cells.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00622-02 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Measurement of Binding of Self and Antigenic Peptides to MHC Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Maripat Corr	Medical Staff Fellow	LI, NIAID
	Sergei Khilko	Visiting Associate	LI, NIAID
	Marina Khilko	Special Volunteer	LI, NIAID

COOPERATING UNITS *(if any)*

LMB, NIDDK (E.A.Padlan); A. Lees, J. K. Inman, BCMS, LI, NIAID

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our objectives in this project have been to examine the interaction of self and antigenic peptides with the MHC class I molecule using several different systems and to evaluate these interactions in three dimensional computer modeling studies based on crystallographic MHC structures. Such studies permit us to examine underlying biochemical rules that govern the interaction of antigenic peptides with MHC molecules, a central role in the initiation of the immune response. In addition they provide a framework for understanding the larger question of how proteins bind small molecular weight peptide ligands.

Major accomplishments in the past year include the development of quantitative kinetic assays for measurement of the interaction of peptides with purified MHC molecules in real time, and the extensive analysis of the binding of a large number of peptides. Characterization of self peptides has provided the basis for the production of a large number of molecular models of MHC/peptide complexes and permits the more detailed description of the molecular rules that contribute to stable binding.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00623-02 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Folding and Assembly of MHC Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Randall Ribaudo	Guest Worker/Staff Fellow	LI, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aims of this project have been to develop *in vitro* systems for the study of the assembly and folding of the MHC class I molecules. This has been based on the use of *in vitro* translation and assembly methods to analyze the contribution of the three chains of this complex, the class I heavy chain, β2-microglobulin, and antigenic peptide. Such studies should not only allow an understanding of this critical step in antigen presentation in the immune system, but should form the basis for an understanding of the folding and assembly of complex multimeric proteins in general.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00624-03 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
New Chemical Tools for Immunology and Structural Biology Research

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John K. Inman	Section Chief	LI, NIAID
Others:	Patricia F. Highet	Technician	LI, NIAID

COOPERATING UNITS *(if any)*
DCBD, Lab Pathology, NCI, NIH (H.C. Krutzsch); NIDR, NIH (F.A. Robey)

LAB/BRANCH
Laboratory of Immunology

SECTION
Bioorganic Chemistry Section

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project covers the design, synthesis and use of new reagents and chemical constructs that will meet specific requirements in research projects of collaborating groups or individuals. Research goals fall within the fields of basic and applied immunology and structural biology. For the preparation of conjugates and immunoadsorbents, heteroligation strategies have played a central role our work. In these strategies, two disparate structures are cross-linked without self-polymerization by placing uniquely interacting functions on each component through chemical derivatization. We have favored the use of sulfhydryl and sulfhydryl-selective groups as the paired functions, with cross-linking occurring through formation of stable thioether linkages. New reagents have been developed for the placement of sulfhydryl-selective, haloacetyl (chloro-, bromo- and iodoacetyl) groups onto proteins, peptides and polymers. In particular, we have introduced a reagent ("BBAL") for placing a bromoacetyl moiety at any desired sequence position of a synthetic peptide, so that the resulting peptide may be directly coupled to sulfhydryl-bearing carriers at this designated position. Peptides that are synthesized with both bromoacetyl- and sulfhydryl-bearing (cysteinyl) residues may be self-polymerized to produce immunogens, or cyclized under different conditions to yield conformationally constrained epitopes for grafting onto immunogenic carriers. We have designed and used improved iodoacetylating agents and also reagents for placing protected/activated sulfhydryl groups on oxidized polysaccharide chains of antibodies and other glycoproteins. The terminal sulfhydryl group is spaced from the polysaccharide chain by intervening structures in order reduce steric hindrance to the approach of macromolecular reactants. We have recently designed, synthesized and tested a selective alkylating agent, *N*-isopropyl iodoacetamide (NIPIA), for cysteine side chains in proteins that permits positive identification of Cys residues as a phenylthiohydantoin (PTH) analogue in the course of microsequencing of amino acids in peptide fragments (at ≤ 10 pmole level). The polarity of the new, stable PTH derivative, resulting from the Edman degradation through Cys positions, is such that its HPLC peak falls in an unused portion of the standard chromatogram. This finding solves the major remaining analytical problem in the chemical structure determination of peptides/proteins

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00625-02 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protein Phosphorylation in the Effector Functions of T-Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	F. Redegeld	Visiting Fellow	LI, NIAID
	P. Rabinovich	Visiting Scientist	LI, NIAID
	V. Sverdlov	Visiting Fellow	LI, NIAID
	P. Smith	Research Associate	LI, NIAID
	Pearl Chen	Research Associate	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunology

SECTION
Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of our studies is to understand the biochemical pathways and to identify key proteins (enzymes) that are involved in the activation and the effector phase of such important immune responses, like T-cell-mediated cytotoxicity (toward virus-infected cells and toward tumor cells) and in lymphokine secretion (e.g. γ-interferon and Interleukin 2). It is expected, that such knowledge will enable us to selectively manipulate different functional responses of T-cells with specifically designed immunomodulators. We attempt to reach this goal by taking advantage of the (A) extensive knowledge of the structure of well known protein kinases and by (B) developing new approaches for the isolation of novel, functionally important protein kinases.

(A). Role of the cAMP-dependent protein kinase (PKA) in effector functions of T-helper and cytotoxic T-lymphocytes(CTL). The results obtained using molecular-biological approaches and synthetic peptide inhibitors suggest the novel view, that PKA has dual role in the regulation of T-lymphocytes effector functions; it down-regulates protein-synthesis independent responses (cytotoxicity and exocytosis of granules), but is required for the TCR-triggered, DNA transcription and protein synthesis-dependent processes (γ interferon or IL-2 secretion). Thus PKA may serve as an attractive target for the immunomodulation.

(B) Identification of novel ectoprotein kinases in lymphocytes. While pursuing our "extracellular ATP" hypothesis of the cell-mediated cytotoxicity we demonstrated the presence and characterized ecto ATPase(s) and highly active and extracellularly located protein kinases (ectokinases) which we implicated in the T-cell functions using several different inhibitors. These enzymes are unusual because they "look"outside the cell surface while residing in the plasma membrane of lymphocytes. At least two ectokinases with different substrate specificities are described to date: "Casein Kinase II-like" ectoenzyme that phosphorylates Ser/Thr in the negatively charged amino acid environment and "Histone-like" kinase with specificity toward basic amino acid surrounded Ser/Thr. These enzymes are now purified and attempts are being made to obtain the amino acid sequences of tryptic peptides to allow the cDNA cloning. Since we also identified PPIA class of ectoprotein phosphatases these data point on the presence of the complete phosphorylation/dephosphorylation system on the surface of lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00626-02 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inhibitors of Protein Kinases (or Phosphatases) as Immunomodulators

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	P. Rabinovich	Visiting Scientist	LI, NIAID
	H. Sugyiyama	Visting Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunology

SECTION
Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our ongoing *in vivo* and *in vitro* studies of functionally important cellular populations and of the role of individual proteins in effector functions of T-lymphocytes have obvious immunopharmacological implications since they identify the cell types and key proteins that should be targeted for efficient immunomodulation when there is a clinical need to inhibit or to enhance the intensity of the effector phase of the immune response.

A. Synthetic peptides inhibitors/pseudosubstrates of protein kinases as modulators of T-lymphocyte responses. We tested if it is possible to use peptides which mimic pseudosubstrate sequences or inhibitory domains of known protein kinases and demonstrated that such peptides are able to block functional responses of T-cells. Some peptides were only inhibitory, while PKA-inhibiting synthetic peptide was able to enhance the TCR-triggered IL2 production of T-cells when directly delivered in the cytoplasm by hypotonic shock. The advantages of this synthetic peptide-based strategy for immunomodulation include the possibility of the rational design of individual peptides according to the defined properties of the targeted enzymes, achieving both biochemical and, hopefully, cell-specific specificity of novel inhibitors.

B. The differential modulation of cytotoxic T cells effector functions. During the systematic studies of the effects of different enzyme inhibitors, anti-sense mRNA oligos and of different synthetic peptide inhibitors on lymphocyte functions we succeeded in demonstration of the possibility to simultaneously inhibit some functions (e.g. γ-interferon secretion) and to enhance other functions of the same cell (e.g. the cytotoxic "lethal hit" delivery and granule exocytosis) by TCR-triggered CTL by interfering with the activities of serine/threonine PP2A phosphatase and of protein kinase A. This forms the basis for the development of the immunomodulating reagents which will selectively affect the partial reactions of the immune response.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00627-02 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulatory Events in T Cell Development in the Thymus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	Li Yin	Visiting Fellow	LI, NIAID
	J. Züniga-Pflucker	Jane Coffin Childs Fellow	LI, NIAID
	Y.-N. Chang	Visiting Student, Cambridge Univ.	LI, NIAID
	Di Jiang	Grad. Student - George Wash Univ.	LI, NIAID

COOPERATING UNITS *(if any)*

Metabolism Branch, NCI, Dr. Louis Staudt

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Increasing evidence suggests important events in T cell maturation in the thymus are governed by specific gene regulation. In the thymus, precursor cells undergo a complex set of developmental events and emerge as mature T lymphocytes capable of specific antigen recognition. We have found that there is a key developmental phase during the double negative stage of thymic development in which the cells resemble antigen-activated mature cells. We have made preparations of day 14/15 triple negative fetal thymocytes that exhibit the activated T lymphocyte markers CD25, ICAM-1, Ly-6A/E, CD44, and HSA and are rapidly proliferating as evidenced by flow cytometric examination of BrdU incorporation. We found that the gene regulators NF-κB, the NF-κB p50 homodimer complex, NF-AT, oct-1, oct-2, AP-1, and SRF, are all were active in these early thymocytes. Whereas the octamer factors and SRF persist during ontogeny, NF-κB, NF-AT, and AP-1 decrease and are undetectable in the adult thymus. Thus, transcriptional events that are usually triggered by antigen stimulation in mature T cells take place early in thymic ontogeny in the absence of the T cell receptor. Our analysis suggests that there are striking regulatory similarities but also important differences between the activation processes that take place in antigen-stimulated mature T cells and thymic progenitor cells. We are now developing an in vitro system that recapitulates early steps in T cell differentiation to determine the molecular signals required. As part of this effort we are beginning to produce targeted mutations in signalling molecule genes to determine their effect on thymic development.

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00628-02 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigen-Induced Apoptosis (Propriocidal Regulation) of Mature T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	S. Boehme	Postdoctoral Fellow	LI, NIAID
	Jeffrey Critchfield	HHMI Scholar - UC-San Francisco	LI, NIAID
	A. Mermelstein	Medical Student - New York Univ.	LI, NIAID
	S. Batra	Summer Student - Harvard	LI, NIAID
	Lixin Zheng	Visiting Fellow	LI, NIAID
	L. Chen	Summer Student - MIT	LI, NIAID

COOPERATING UNITS *(If any)*
Neuroimmunology Unit, NINDS, Dr. Michael Racke; Laboratory of Immunology, NEI, Dr. Rachel Caspi; Dept Biotechnology, Univ. Washington, Seattle, Dr. Joan Goverman; Immunology Division, Alexion Pharmaceutricals, Dr. Lou Matis

LAB/BRANCH
Laboratory of Immunology

SECTION
Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have found that stimulation of the antigen receptor of mature T cells following exposure of the cells to mitogenic lymphokines such as IL-2 leads to the induction of programmed cell death or apoptosis (propriocidal regulation). This process can lead to the deletion of specific antigen-reactive T cells without affecting bystander cells. We are interested in two aspects of this effect: 1) the molecular events that take place, and 2) the possibility of using this mechanism to pharmacologically induce the elimination of T cells involved in disease processes. Our results indicate that antigen-induced apoptosis can only occur at specific stages of the cell cycle. We can demonstrate that appropriate immunization will lead to the suppression of immune responsiveness and more recently that the specifically reactive T cells have been eliminated.

Several types of diseases that arise from the over-stimulation of the immune system including: allergies, autoimmune disorders such as multiple sclerosis and rheumatoid arthritis, and the rejection of tissue grafts. Activated T cells are the driving force behind the destructive immune response in these diseases. Current therapies involve drugs such as steroids and cyclosporin A that impair, in a general way, T lymphocyte function. Therefore, one serious side effect of these therapies is to increase susceptibility to infections. We have discovered that the cytokine IL-2 is able to cause the death of T cells that are specifically stimulated through their antigen receptor. This previously unknown property of IL-2 should allow the specific elimination of certain receptor-bearing T cells which could form the basis for new clinical applications of IL-2. Our results thus far indicate that mature T cell deletion by the propriocidal mechanism can be useful for ameliorating disease in mouse models for multiple sclerosis and autoimmune eye disease. Other disease models are currently under investigation. This method for treatment of T cell mediated diseases should avoid the severe immunosuppression which is a side-effect of current therapies.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00630-02 LI

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of γδ T cells in Immunoregulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.M. Shevach	Section Head	LI, NIAID
Others:	C. Brando	Visiting Associate	LI, NIAID
	D. Salomon	Guest Worker	LI, NIAID
	A. Bonomo	Visiting Fellow	LI, NIAID
	C. Mojcik	Medical Staff Fellow	ARB, NIAMS

COOPERATING UNITS *(if any)*
Biological Resources Branch, NIAID, NIH (G. Kikuchi, J. Coligan)
Department of Medicine, Hebrew University School of Medicine, Jerusalem, Israel (D. Eilat)

LAB/BRANCH
Laboratory of Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

γδ T lymphocytes represent a minor subpopulation of T cells which are found only in small numbers in peripheral lymphoid organs, but which represent the predominant T cells in may epithelial sites. Although the overall structure of the γδ T cell receptor (TCR) appears to resemble that of the αβ TCR, very little is known about the nature of the antigen and the presentation molecules recognized by the γδ TCR. Although both αβ and γδ T cells are derived from precursors that enter the thymus, γδ T cells appear in the thymus on day 14-15 of gestation, 2-3 days before the appearance of αβ T cells. To further explore the possible contribution of γδ T cells to thymocyte development , we transferred γδ T cells to SCID mice which lack T or B cells. Transfer of γδ T cells resulted in the appearance of CD3-CD4+CD8+ thymocytes. These results suggest that one role of γδ T cells is to promote the development of CD4 and CD8 expression on precursor cells in the thymus. Our studies on thymocyte development have been expanded to elucidate the pathogenesis of autoimmune disease which is observed when mice are thymectomized on day 3 of life. We have demonstrated that the lymph nodes of 3 day old mice contain 20-50% CD4+CD8+HSA+ T cells which express low to intermediate levels of CD3. These double positive T cells are thymus derived and cannot be detected after day 10 of life. These studies suggest that the thymus normally prematurely exports cells to peripheral lymphoid tissue and that removal of the thymus leads to the uncontrolled differentiation of these cells in the periphery which may result in autoimmune disease. Our previous studies had suggested that members of the integrin family of cell surface antigens play an important role in the activation of γδ T cells in vitro. To further investigate the contribution of the integrins to thymocyte development, we analyzed human thymocytes for expression of integrin receptors as well as their ability to adhere to extracellular matrix proteins. A subpopulation of CD4+CD8+ TCR^{low} human thymocytes was shown to spontaneously adhere to fibronectin and to express high levels of the fibronectin binding integrins α4β1 and α5β1; however, their adherence to fibronectin could only be inhibited by anti-α4β1 antibodies. Fibronectin non-adherent thymocytes expressed lower levels of CD4/CD8 and higher levels of CD3, but levels of α4β1 and α5β1 identical to the fibronectin adherent population. These studies raise the possibility that activation and deactivation of integrin function may play an important role in thymocyte development.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

LABORATORY OF INTRACELLULAR PARASITES
Rocky Mountain Laboratories
Hamilton, Montana
1993 Annual Report
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Annual Report
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1992 to September 30, 1993

Research Highlights

Scientists in the Laboratory of Intracellular Parasites (LICP) study infectious diseases caused by the obligate intracellular bacterial pathogens *Chlamydia trachomatis* and *Rickettsia rickettsii*. The laboratory focuses on two general areas of study: (i) biology of host parasite interactions, and (ii) the hosts immune response to these obligate intracellular pathogens. Molecular biology and genetic approaches are being used to characterize parasite surface components in relation to pathogenicity and for the study of parasite developmental biology. Parasite surface components that function in attachment to eucaryotic host cells have been identified and are being used for the rationale design of experimental recombinant or sub-unit vaccines for the prevention of human diseases caused by these intracellular pathogens. This work has led to the design of a synthetic peptide immunogen that shows considerable promise as an experimental prototype vaccine against chlamydial sexually transmitted diseases. Animal models are being used to define the role of host immunity in mediating chlamydial disease pathology. Chlamydial antigens that evoke deleterious immune responses have been identified and immunologic mechanisms that mediate immunopathology are being studied. Scientists in LICP have identified chlamydial gene(s) that function in regulating the intracellular differentiation of chlamydial replicative and infective forms. A more precise understanding of the genetic and biologic mechanisms that regulate chlamydial intracellular growth offers potential for the development of new non-immunological based therapeutic strategies for the control of chlamydial diseases.

Immunology of Intracellular Parasites Section: There are two major research themes within the Immunology Section; (i) development of animal models for the study of protective and pathologic immune responses to chlamydial infection, and (ii) the development of a safe and efficacious chlamydial vaccine. Projects involving vaccine development have been ongoing since the inception of LICP and have focused on the design of synthetic oligopeptides immunogens corresponding to key T-helper and neutralizing B-cell epitopes of the chlamydial major outer membrane protein (MOMP). Accomplishments in this area includes the molecular mapping of MOMP T-helper and neutralizing epitopes, synthesizing these epitopes as co-linear peptide immunogens, and defining the peptides immunogenicity and protective efficacy in animal models of chlamydial infection. Prototype peptide vaccines have been shown to target the production of functional anti-chlamydial neutralizing antibodies and to have a high potential for wide MHC recognition. Preliminary vaccine efficacy studies in subhuman primates indicate that systemic immunization provides partial protection against chlamydial disease but does not prevent chlamydiae from colonizing mucosal surfaces. Future research in this area will focus on targeting the peptide immunogens to evoke mucosal as well as systemic neutralizing antibodies. This work will include encapsulation of peptide immunogens into biodegradable microspheres, and the construction of recombinant poliovirus and *E. coli* heat labile enterotoxin vectors expressing targeted chlamydial neutralizing epitopes.

Host-Parasite Interactions Unit: The overall focus of the Host-Parasite Interactions Section has been shifted over the last two years toward more basic biological processes involved in intracellular growth of *Rickettsia spp.*, *Chlamydia trachomatis*, and *Coxiella burnetii*. The research emphasis can be categorized into two broadly defined areas; control of gene expression by intracellular parasites and host cellular responses to infection. Within these broad categories, specific projects are assigned. Our interest in gene regulation currently focuses on the role of some recently identified histone H1 homologs in control of the developmental cycles of *Chlamydia trachomatis* and *Coxiella burnetii*. Histone H1 homologs are rare among prokaryotes, *C. trachomatis* possesses two elementary body specific proteins with primary amino acid sequence homology to eukaryotic H1. These chlamydial histone homologs appear to play a major role in establishment of nucleoid structure as well as in control of gene expression. Investigations into the host's contributions to intracellular bacterial growth emphasize the role of signal transduction pathways induced by parasite attachment and how the resulting second messengers may trigger internalization or modulate the intracellular environment. One of the more dramatic examples of subversion of host constituents to effect a pathogenic mechanism is the mobilization of actin to promote intracellular movement and cell to cell spread of spotted fever group rickettsiae.

ADMINISTRATIVE REPORT

The LICP is divided into two sections: (i) the Host-Parasite Interactions Unit and (ii) the Immunology of Intracellular Parasites Section. The Host-Parasite Section is headed by Dr. Ted Hackstadt and the Immunology Section by Dr. Harlan Caldwell. Scientists working in the Host-Parasite Section include: Dr. Robert Gilmore (Staff Fellow), Dr. Paul Policastro (Senior Staff Fellow), Dr. Clifton Barry (IRTA), Dr. Tim Brickman (IRTA), Dr. Robert Heinzen (IRTA), and Dr. Sita Awasthi (Visiting Fellow). Scientists working in the Immunology Section include: Dr. Richard Morrison (Research Microbiologist), Dr. Hua Su (Visiting Associate), Dr. Scott Manning (Staff Fellow), Dr. Dan Rockey (IRTA), Dr. Todd Cotter (IRTA) and Dr. Yuan Ying (Visiting Associate). Dr. Yuan joined the laboratory this fiscal year on a loaner FTE. The total FTE and non-FTE scientific slots allotted to LICP are occupied. Summer students working in LICP include: Jenifer Maloney (Pre-IRTA) and Melissa Reeves (Clerical). The following people were invited, by LICP staff, to present seminars: Dr. John H. Eldridge (University of Alabama at Birmingham), Dr. Vojo Deretic (University of Texas), Dr. Lewis G. Tilney (University of Pennsylvania), Dr. Chander Raman (Loyola University of Chicago), Dr. David Kaslow (LMR/NIAID), Dr. Yvonne Pannekoek (University of Amsterdam) and Dr. Kim Janda (Scripps Institute)

HONORS AND AWARDS

NIH Honor Awards:

R. Morrison - NIH Award of Merit

Journal Editorial Boards:

H. Caldwell - Infection and Immunity

Ad Hoc Reviewers:

H. Caldwell - Cell
Journal of Bacteriology
Journal of Infectious Diseases
Journal of Immunology
Proceedings of the National Academy of Sciences
Microbiol Pathogenesis

T. Hackstadt - Infection and Immunity
Journal of Bacteriology
Molecular Microbiology

R. Morrison - Journal of Clinical Investigation
Infection and Immunity
Journal Infectious Diseases
Journal of Immunology

Professional Posts:

H. Caldwell - Faculty affiliate, Division of Biological Sciences (Microbiology), University of Montana, Missoula, Montana.

R. Morrison - Faculty affiliate, Division of Biological Sciences (Microbiology), University of Montana, Missoula, Montana.

Elected Post:

T. Hackstadt - Councilor-at-large, American Society for Rickettsia and Rickettsial Diseases.

R. Morrison - Chair-elect, Division of General Medical Microbiology, American Society for Microbiology.

Invited Lectures and Participation in Meetings and Symposia:

C. Barry - The Cell and Molecular Biology of Bacterial Host Cell Interactions, Monterey, California
Bristol-Meyers Squibb, Princeton, New Jersey

T. Brickman - American Society for Microbiology, Atlanta, Georgia

T. Cotter - American Society for Microbiology, Atlanta, Georgia

R. Gilmore - American Society for Microbiology, Atlanta, Georgia
Seminar, Oklahoma City, Oklahoma
Seminar, Colorado Springs, Colorado
Seminar, Iowa City, Iowa

T. Hackstadt - The Cell and Molecular Biology of Bacterial Host Cell Interactions, Monterey, California

R. Heinzen - Washington State University, Pullman, Washington
American Society for Microbiology, Atlanta, Georgia

S. Manning - American Society for Microbiology, Atlanta, Georgia

R. Morrison - Seminar, Indianapolis, Indiana
Seminar, San Antonio, Texas
American Society for Microbiology, Atlanta, Georgia

H. Su - Combined Vaccines and Simultaneous Administration: Current Issues and Perspectives, Bethesda, Maryland
Microbial Pathogenesis and Immune Response, Orlando, Florida

Other Activities:

H. Caldwell - NIAID Study Section, Bacteriology and Mycology, Ad hoc member
National Advisory Allergy and Infectious Diseases Council
NIAID Promotion and Tenure Committee

T. Hackstadt - NIAID Study Section, Bacteriology and Mycology, Ad hoc member
NIAID, RML, Safety Committee

R. Morrison - NIAID Study Section, Bacteriology and Mycology, Ad hoc member
NIAID Study Section, Tropical Medicine and Parasitology, Ad hoc member
Merit Review Board, Department of Veterans Affairs, Ad hoc member
Welcome Trust, London, England, Ad hoc member
Swiss National Science Foundation, Ad hoc member
NIAMS Study Section, Arthritis and Musculoskeletal and Skin Diseases, Ad hoc member
NIAID, RML, Animal Care and Use Committee
NIAID, RML, Biosafety Committee

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** ZO1 AI 00216-13 LICP

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Chlamydial Vaccine Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.D. Caldwell	Chief	LICP, NIAID
Others:	H. Su	Visiting Associate	LICP, NIAID
	D. Rockey	IRTA	LICP, NIAID
	S. Stewart	Microbiologist	LICP, NIAID
	J. Simmons	Bio. Lab Tech.	LICP, NIAID

COOPERATING UNITS *(if any)*

Dr. Andrew Murdin, Connaught Laboratories, Willowdale, Ontario, Canada

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Immunology of Intracellular Parasites

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	**PROFESSIONAL:**	**OTHER:**
4.3	2.4	1.9

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The overall objective of this work is the design and development of a synthetic peptide vaccine for the prevention or control of sexually transmitted diseases (STDs) caused by *C. trachomatis*. Chlamydial STDs are caused by multiple serovars and therefore an efficious vaccine must be capable of evoking a broadly cross protective response that can be targeted to evoke local (mucosal) immunity. The membrane protein (MOMP) is the most promising antigen for the development of a chlamydial vaccine. We have identified and mapped at the molecular level T-helper and B-cell neutralizing epitopes of the MOMP that are antigenically common among those *C. trachomatis* serovars that are important etiological agents of chlamydial STDs. These key epitopes of the MOMP were co-linearly synthesized as a large chimeric T:B cell oliopeptide and its immunogenenic properties studied in mice and sub-human primates. The oligopepitde immunogen was immunogenic in many congenic mouse strains differing at H-2, and effectively targeted the production of high titered broadly cross-neutralizing anti-chlamydial antibodies. The oligopeptide was also highly immunogenic in sub-human primates evoking a strong heterotypic serum neutralizing antibody response. Parenteral immunization of primates did not produce local neutralizing antibodies and did not confer protection against cervical challenge with chlamydiae. Future work will focus on alternate immunization stratagies with the oligopeptide designed to induce mucosal immunity. These studies will include encapsulation of the oligopeptide into biodegradable/biocompatable microspheres or liposomes, cross-linking of the peptide to cholera toxin B-subunits, and the construction of recombinant attenuated poliovirus vectors expressing targeted chlamydial neutralizing epitopes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00519-06- LICP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenesis of Chlamydial Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.P. Morrison	Microbiologist	LICP, NIAID
Others:	K. Lyng	Chemist	LICP, NIAID

COOPERATING UNITS *(if any)*

Univ. Toronto, Ontario, Canada (R. Inmam); Univ. Wisconsin Med. Sch., Madison, WI (G.Byrne, W.Beatty); New York Hosp.-Cornell Med. Ctr., New York, NY (S. Witkin); University of Montana, Division of Biological Science, Missoula, Montana (Y. Ying). Indiana Univ. School of Medicine, Indianapolis, Indiana (R.Jones, J.Arno).

LAB/BRANCH

Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION

Immunology of Intracellular Parasites

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Infections caused by the obligate intracellular bacterium *Chlamydia trachomatis* are among the most prevalent causes of genital and ocular diseases worldwide. Chlamydial infections can progress to chronic inflammatory sequelae that lead to infertility or blindness. The immune mechanisms that augment chronic inflammation are ill-defined, but repeated exposure to chlamydial antigens is thought to contribute to the disease process. Although the importance of repeated infection in the development of the chronic inflammatory sequelae of chlamydial disease has been established, persistent infection might also serve as a source of antigen for the chronic inflammatory responses. The primary objective of our studies this past year have been to develop and define an *in vitro* cell culture model of persistent chlamydial infection. Understanding persistent chlamydial *in vitro* may help provide us with a better understanding of the pathogenesis of chlamydial disease *in vivo*.

An *in vitro* cell culture system was used to study the effect interferon-γ (IFN-γ) on *Chlamydia trachomatis* growth and differentiation. The effect of IFN-γ was dose dependent. High concentrations (2 ng/ml) of IFN-γ completely inhibited chlamydial growth and differentiation, whereas persistent infection was established when chlamydia infected cells were cultured with 0.2 to 0.5 ng/ml IFN-γ. Persistent infection was characterized by the development of noninfectious atypical chlamydial forms from which infectious progeny could be recovered only when IFN-γ was removed from the culture system. Analysis of persistently infected cells by immunofluorescent microscopy and immunoblotting with specific antibodies revealed that the atypical chlamydial forms had near normal levels of the 60-kDa heat shock protein, an immunopathologic antigen, and a paucity of the major outer membrane protein, a protective antigen. If IFN-γ causes similar events to occur *in vivo*, then persistently infected cells could augment the pathogenesis of the chronic inflammatory sequelae that follow chlamydial infection by serving as depots of antigen capable of stimulating a sustained inflammatory response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00567-03 LICP

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Biology of Host-Parasite Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Hackstadt	Expert	LICP, NIAID
Others:	P.F. Policastro	Senior Staff Fellow	LICP, NIAID
	R.D. Gilmore	Staff Fellow	LICP, NIAID
	R.A. Heinzen	IRTA	LICP, NIAID
	S. Awasthi	Visiting Fellow	LICP, NIAID
	R.E. Mann	Bio Lab Tech.	LICP, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Host-Parasite Interactions Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The projects described below are new initiatives that represent an effort to refocus the Section's activities to emphasize molecular studies of more basic biological processes involved in prokaryotic intracellular parasitism. The current objective of these projects is defining the initial signal transducing event, G-proteins or tyrosine kinase, that may be coupled to the actual parasite receptor and exploring how these cellular regulatory cascades may modify the intracellular environment to benefit the parasite. Host cell responses to *Chlamydia trachomatis* infection detected so far include components of signal transduction pathways such as phospholipase C, phospholipase A2, and protein kinase C activities as well as calcium mobilization. One of the more dramatic examples of subversion of host constituents to effect a pathogenic mechanism is the mobilization of actin to promote intracellular movement and cell to cell spread of spotted fever group rickettsiae. Staining of *Rickettsia rickettsii* infected VERO cells with rhodamine phalloidin demonstrated unique actin filaments associated with one pole of intracellular rickettsiae. Biochemical evidence that actin polymerization plays a role in movement was provided by demonstrating that exocytosis of *R. rickettsii* from infected cells was inhibited by cytochalasin D. A recent finding of a histidine kinase-like gene related to prokaryotic two-component regulatory systems may provide some unique approaches toward identification of environmental signals that regulate specific genes intracellularly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00612-03-LICP

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression and Analysis of Recombinant Chlamydial Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Scott Manning	Staff Fellow	LICP, NIAID
Others:	H. Su	Collaborative Scientist	LICP, NIAID
	S. Stewart	Microbiologist	LICP, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Immunology of Intracellular Parasites

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The focus of this project is the use of recombinant genetic methods to express epitopes of the major outer membrane protein of *Chlamydia trachomatis* in ways which will facilitate the induction of mucosal immune responses to these epitopes. Two methods are being used.

1) The epitopes are being expressed on surface exposed regions of poliovirus. Attenuated poliovirus strains may serve as safe and effective infectious vaccine vectors for stimulation of mucosal immunity.

2) The epitopes are being genetically fused to the *E. coli* heat labile toxin (LT). LT has the unusual property of breaking oral antigen tolerance and thus, functions as a potent mucosal adjuvant.

The antigenic and immunogenic properties of epitopes expressed in each of these systems are being characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00631-02 LICP

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Biology of Intracellular Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Hackstadt	Expert	LICP, NIAID
Others:	C.E. Barry	IRTA	LICP, NIAID
	T. Brickman	IRTA	LICP, NIAID
	J.D. Sager	Bio. Lab. Tech.	LICP, NIAID

COOPERATING UNITS *(If any)*
None

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Immunology of Intracellular Parasites Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.4	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Modification of DNA structure by histone-like proteins appears to be a central regulatory mechanism governing the complex life cycle of *Chlamydia trachomatis*. Histone H1 homologs are rare among prokaryotes, *C. trachomatis* possesses two EB specific proteins with primary amino acid sequence homology to eukaryotic H1. These histone homologs, termed Hc1 and Hc2, are expressed only during the late stages of the chlamydial life cycle concomitant with the reorganization of RBs into EBs and appear to play a major role in establishment of nucleoid structure as well as in control of gene expression. Expression of Hc1 in *E. coli* results in a compaction of the chromatin that ultrastructurally resembles the nucleoid reorganization which occurs late in the chlamydial developmental cycle. Hc2 expression in *E. coli* also condenses the chromatin although no comparable structure is observed in either EBs or intermediate developmental forms. In contrast to the electron-dense spheres produced by Hc1, Hc2 expression produces nucleoids that have a distinct, coil-like appearance. Hc2 is enriched in isolated nucleoids from Hc2 expressing *E. coli* and protects the isolated DNA from nucleolytic digestion. Hc1 expression in *E. coli* is self-limiting and produces a global termination of transcription, translation, and replication at concentrations equivalent to that of chlamydial elementary bodies. We have proposed that association of Hc1 with DNA at levels below that necessary to condense the nucleoid may exert more specific regulatory effects through modification of DNA structure/topology to influence promoter activity and gene expression. Alteration of DNA topology in response to environmental signals appears to play an underlying role in regulation of gene expression in bacteria. Hc1-mediated effects on DNA topology may thus play a significant role in the regulation of gene expression during chlamydial development. Understanding the controlling mechanisms for gene expression and differentiation may suggest unique methods of interrupting parasite replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** ZO1 AI 00672-01 LICP

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mucosal Immunity to Chlamydial Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.D. Caldwell	Chief	LICP, NIAID
Others:	T. Cotter	IRTA	LICP, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Immunology of Intracellular Parasites

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.5	**PROFESSIONAL:** 1.5	**OTHER:** 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The goal of this project is to directly determine whether Chlamydia specific secretory IgA (sIgA) plays a role in protection from, or resolution of, chlamydial infection of the mouse genital tract. In order to address this question we will 1) produce murine hybridomas that secrete polymeric IgA (pIgA) monoclonal antibodies (mAbs) specific to chlamydial surface components, and 2) determine their ability to protect against chlamydial genital tract infection in a murine model. mAbs will be administered to naive animals by subcutaneously implanting a mAb secreting hybridoma in the backs of syngeneic mice (backpack tumor system). This approach is made possible by the fact that systemically delivered pIgA is specifically complexed with secretory piece and transported to mucosal surfaces as sIgA, whereas monomeric IgA (mIgA) and IgG are not. To date, three murine hybridomas have been generated that produce pIgA specific for the surface of *Chlamydia trachomatis* MoPn. These hybridomas resulted from fusion experiments using mesenteric lymph node derived lymphocyte populations that were enriched for IgA surface positive cells. Using pIgA and IgG secreting hybridomas, we are currently conducting a pilot experiment to characterize the backpack tumor system in our laboratory. Preliminary data indicate that the take rate for tumor development is >80%, that high levels of mAb are produced by such tumors, and that tumor growth rates vary significantly between tumor cell lines. With this information, we can now evaluate the role of anti-chlamydial sIgA in protection against chlamydial colonization of the genital tract mucosae as well as its role in resolving established infections of the genital tract. The same collection of mAbs will allow for future studies that compare *in vivo* protection with *in vitro* neutralization of chlamydial infectivity, as well as, investigations of mucosae specific IgA antibody dependent cellular cytotoxicity as a potential mechanism of protective immunity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AI 00693-01 LICP

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenic Mechanisms of *Mycobacterium tuberculosis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Caldwell	Chief	LICP, NIAID
Others:	C.E. Barry	IRTA	LICP, NIAID

COOPERATING UNITS *(if any)*
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SECTION
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INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

This is a new initiative within LICP to begin a program in mycobacterial pathogenesis. To date the only effort on this project has centered on equipping a P-3 facility in which to grow the virulent organism and designing potential projects to begin work. A description of the proposed course of work follows; **(1) Elucidation of the immediate enzyme target of Isoniazid action.** Isoniazid (INH) represents the front-line defense against tuberculosis yet its mode of action and the reason for its mycobacterial specificity is not understood. INH has a quantitative effect on mycolic acid biosynthesis and as a consequence an effect on cell-wall integrity. This proposal will attempt to identify the enzyme target of INH sensitivity by photoaffinity-labelling of the enzyme involved with radiolabelled INH. INH has also been proposed to act thru interference with nicotinamide cofactor activity. This will be explored through differential competitive photolabelling with 2-azido-NAD$^+$/H. The availability of a specific enzyme target will allow determination of the biosynthetic step affected and will allow a direct assay to guide the development of anti-mycobacterial chemotherapeutics. **(2) Regulation of cell-wall biosynthesis in *Mycobactrium tuberculosis* H37Rv.** In *E. coli* fatty acid metabolism/biosynthesis is coordinately regulated by FadR which has a dual role as both repressor of transcription of catabolic enzymes and activator of transcription of anabolic pathways. FadR binds to long-chain fatty acyl coenzyme A esters within the cytoplasm and undergoes a conformational change which relieves repression of the catabolic enzymes necessary for fatty acid degradation. The regulation of the biosynthesis of complex lipid structures in mycobacteria is essentially unknown but perhaps operates using similar mechanisms. This research will investigate the effect of various lipids and lipid precursors on total incorporation of acetyl-CoA into lipid fractions specific for the cell-wall. Cytoplasmic proteins which bind to labelled target lipids will be identified and their role in regulation of lipid metabolism will be explored. **(3) Determination of the mechanism of formation and function of cyclopropanated mycolic acids in the cell-wall core of Mycobacterium tuberculosis.** Cyclopropanated mycolic acids occur at the interface of hydrophobic domains in the mycobacterial cell-wall. The function of these modifications may be structural or they may serve to deactivate potential targets for oxidative attack by host organisms. The proposed experiments will attempt to demonstrate cyclopropanation of precursors to mycolic acids in purified cell-free extracts and utilize this activity to purify, clone and sequence the enzyme from *M. tuberculosis*. Targeted gene disruption of this activity will allow a direct assesment of the role of this structure in mycobacterial virulence. **(4) Distribution and function of fatty acid synthase-like complexes in *Mycobactrium tuberculosis* H37Rv.** Many of the wide variety of complex lipids encountered in the mycobacterial cell-wall appear to be biosynthetically related to fatty acids. The biosynthetic mechanism of fatty acid formation is well understood for most organisms and involves common enzymatic modules. Using motifs common to these sytems which catalyze biological "Claisen-condensations" we will attempt to identify and analyze the genetic loci responsible for the biosynthesis of different families of mycobacterial lipids.

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Summary Statement
LABORATORY OF INFECTIOUS DISEASES
National Institute of Allergy and Infectious Diseases
October 1, 1992 to September 30, 1993

RESPIRATORY VIRUSES SECTION

Respiratory Syncytial Virus (RSV): Rescue of Incomplete RSV vRNA From Viral cDNA. It has not been possible to produce infectious virus from cDNA of the viral genome of any non-segmented negative strand RNA virus. The ability to directly introduce changes into virus via cDNA mutagenesis would have obvious, important applications to studies of molecular biology and pathogenesis as well as to vaccine development. Major complications are that: (i) homologous recombination is essentially nonexistent in this situation, and (ii) the negative-sense viral RNA (vRNA) is not directly infectious as naked RNA. In virions or intracellularly, vRNA is always found tightly encapsidated in a ribonucleoprotein core that contains the viral proteins necessary for transcription and replication and that probably is the minimum unit of infectivity. Thus, the production of virus from cDNA will require combining synthetic vRNA with appropriate viral proteins for the assembly of biologically-active nucleocapsids. Considerable progress has been achieved for a segmented negative strand RNA virus, influenza A virus. It is now possible to introduce individual DNA-encoded synthetic gene segments into infectious influenza A virus, although a complete synthetic virus has not yet been produced. These efforts at gene rescue were facilitated by the ability of segmented viruses, such as influenza A, to undergo gene segment reassortment; *i.e.*, to readily accept individual heterologous gene segments. The nonsegmented viruses such as RSV pose a special challenge because the large vRNA must be manipulated in toto.

The large size and complexity of RSV vRNA is such that, even with successful rescue, it may be technically difficult to make the many mutations which will be required for certain projects. Therefore, an alternative approach was developed based on short synthetic vRNA analogs containing one or more marker genes. The recent successful rescue of these synthetic analogs: (i) supplies a powerful system for structure-function studies of *cis*-acting RNA sequences and *trans*-acting viral proteins, (ii) supports the view that it will indeed be possible to produce biologically active full-length vRNA, and (iii) provides the means for developing and optimizing conditions for rescue.

cDNA was constructed to encode an RSV vRNA analog, RSV-CAT, which contains a large internal deletion that eliminates all of the viral genes. The viral genes were replaced by the chloramphenicol acetyl transferase (CAT) reporter gene flanked by the so-called RSV gene-start and gene-end sequence motifs, which are thought to be transcriptive signals. This engineered transcription cassette was flanked in turn by the extragenic 3' (leader region) and 5' (trailer region) vRNA termini. The RSV-CAT cDNA was placed in a plasmid vector under the control of a promoter for bacteriophage T7 RNA polymerase and was flanked on the other end by an Hga1 site for linearization (in the DNA template) or by a self-cleaving ribozyme motif (in the RNA transcript). Transcription *in vitro* of Hga1-linearized plasmid yielded the RSV-CAT vRNA analog, which is negative sense and contains the correct, authentic vRNA 3' and 5' ends. The prototype analog contains 86 nucleotides (nt) and 179 nt of authentic RSV sequence at the 3' and 5' ends, respectively, and is 935 nt in length compared to 15,222 nt for the complete, authentic vRNA.

Upon transfection into RSV-infected cells, the RSV-CAT analog was encapsidated by proteins provided by the helper virus and then "rescued" such that it was amplified, expressed, and packaged into an infectious form which could be passaged onto fresh cells to initiate another cycle of expression and packaging. The packaged RSV-CAT was insensitive to RNAse but was sensitive to RSV-specific antibodies, suggesting that it had been packaged in an RSV envelope. In later experiments it was shown that the positive- and negative-sense versions of RSV-CAT were equally active in rescue, and no differences were observed between the two in their rescuability at various times post-infection.

A similar experimental system was developed for rescue of human parainfluenza virus type 3 (PIV3) synthetic RNA analogs. The *cis*-acting PIV3 signals for amplification, expression and packaging of the synthetic viral analog were similar to those demonstrated for RSV. But, a number of important differences were noted which appear to be indicative of the phylogenetic distinctiveness of PIV3 and RSV.

Novel Molecular Biological Approach to Development of Live Attenuated RSV Vaccine. A novel approach to RSV vaccine development involves efforts within LID to develop methods for producing infectious RSV from its full-length cDNA. This would make it possible to produce and characterize attenuated vaccine strains by direct genetic engineering. To date, a cDNA-encoded vRNA analog which is 49.3% of full-length RSV vRNA has been successfully "rescued". This analog includes a foreign reporter gene and the large RSV L gene (~42% of the viral genome) under the control of RSV transcriptive signals. Standard infectious RSV was used as helper to provide proteins to complement the cDNA-encoded vRNA analog. This analog was packaged into infectious particles and was passaged five times sequentially in culture with undiminished efficiency. This observation provides support for the view that a complete, nondefective cDNA-encoded vRNA can be rendered biologically active so as to yield infectious virus.

Chemical Mutagenesis Approach to Development of Live Attenuated RSV Vaccine. Significant progress has been made in the development of a live attenuated RSV vaccine that is being pursued under an LID/NIAID-Wyeth-Ayerst Research CRADA. Previously developed RSV mutants that were incompletely attenuated for susceptible infants were subjected to chemical mutagenesis in an attempt to introduce additional attenuating mutations and a large series of more attenuated derivatives were generated. These further attenuated RSV mutants, which are thought to contain at least two to four attenuating mutations, were evaluated *in vitro* and *in vivo* in mice and chimpanzees. This extensive evaluation led to the identification of six candidate RSV vaccine strains that exhibited a spectrum of degrees of attenuation *in vivo*, with at least three distinct levels of restriction easily discernable. In each instance, attenuation in animals was greater than that observed for the incompletely attenuated parental mutants from which the candidate vaccine strains were derived. The levels of attenuation of the candidate vaccine strains were well within the range which should be appropriate for a human vaccine. In addition, infection of chimpanzees with each of the three highly attenuated RSV vaccine mutants tested thus far, induced almost complete resistance in the lungs to subsequent RSV challenge. These findings form the basis for our decision to proceed with the evaluation of these three candidate vaccine strains, as well as three other similar candidate strains, in humans. Virus suspensions of these six RSV vaccine candidates are being produced under GMP conditions for Phase I-II clinical studies in humans that should allow us to identify one or more mutants that exhibit the optimal levels of attenuation, immunogenicity, protective efficacy and genetic stability.

Human Monoclonal RSV F Glycoprotein Antibody Fabs Cloned From Combinatorial Libraries Constructed in Filamentous DNA Bacteriophage. In collaboration with scientists at The Scripps Research Institute, human monoclonal antibodies to RSV are being developed for: (i) passive immunoprophylaxis of infants and children who are at high risk for serious RSV lower respiratory tract disease, and (ii) immunotherapy of severe RSV disease in all age groups. Two interesting genetically distinct Fab fragments of RSV F glycoprotein-specific IgG were selected from a combinatorial library prepared in a filamentous DNA bacteriophage vector that expresses a single Fab at its tip. These Fabs were shown to have high neutralizing activity for RSV in cell culture. One of these Fabs effected a 5,000-fold reduction in the titer of RSV in the lungs of infected mice following a single intranasal instillation of Fab at the height infection. These observations suggest that human RSV-specific monoclonal antibody Fab fragments grown in *E. coli* may be sufficient to treat serious RSV disease in humans. Alternatively, the Fab encoding sequences will be engineered to produce complete IgG and IgA in mammalian cell-expression systems and this might significantly increase the efficacy of these synthetic antibodies. Also, whole IgG antibodies (*i.e.*, Fc + Fab) constructed from the most active Fabs will be required for prevention of serious RSV disease in high risk individuals because of the need to maintain a high serum antibody level during prolonged periods of passive immunoprophylaxis.

Human Parainfluenza Virus Type 3 (PIV3). PIV3 is an enveloped virus with a non-segmented single stranded RNA genome of negative sense. It is a significant cause of severe lower respiratory tract diseases in infants and young children such as croup, bronchiolitis and pneumonia. The development of a vaccine against disease caused by PIV3 is therefore an important priority. The JS strain of human PIV3 was used as the wild type parent of produce three vaccine candidate live attenuated cold-passaged (*cp*) viruses; namely *cp*12, *cp*18, and *cp*45. In order to develop an understanding of the genetic basis of attenuation of the mutant viruses, analysis of the nucleotide and deduced amino acid sequence of the JS wild type (*wt*) and *cp*12 and *cp*45 mutant viruses was undertaken and has been completed. The most promising attenuated vaccine candidate, the *cp*45 virus, contains 18 nucleotide changes from its JS *wt* virus. The cDNA clones that were employed to sequence the JS *wt* and *cp*45 viruses are being used to construct complete cDNA copies of each of these two viruses that can be used for rescue of infectious virus, a project that is 90% complete for the JS *wt*.

New Strategy for Genetic Engineering of Attenuated Influenza A Viruses. A new strategy for the construction of attenuated influenza A viruses was developed and implemented during the past year. The construction of candidate live attenuated influenza A virus vaccines can be accomplished most efficiently and expeditiously by transfer of attenuating mutant genes from an attenuated donor virus to a new antigenic variant shortly after it emerges and is perceived to pose an epidemic threat. In these instances transfer would be mediated by gene reassortment when the donor virus and the new epidemic virus are allowed to coninfect cells in culture. Recovery of the desired virus reassortant from the mixed progeny of this dual infection is achieved by applying strong selective pressures that favor growth of virus that bears the surface glycoproteins (H and NA) of the new influenza virus antigenic variant and the attenuating gene or genes of the donor virus. The H and NA glycoproteins of the new virus strain must be conserved in the candidate vaccine virus being prepared because these viral surface proteins represent the major protective antigens. This means that transferable attenuating mutations must be restricted to genes that code for internal or non-structural viral proteins.

The new approach involves the use of site-specific mutagenesis to introduce one or more specific mutations into the full length cDNA of a viral gene that codes for an internal or non-structural protein. This is followed by rescue of the full-length RNA transcripts of the mutant cDNA into infectious virus and selection of the desired reassortant virus bearing the mutant gene. The technique for rescue of a synthetic RNA influenza virus gene originally developed several years ago by other scientists could not be employed because its use is limited to the genes coding for the viral surface H and NA glycoproteins. Therefore, it was necessary to develop a new selection system that strongly favored recovery of reassortant viruses bearing the rescued mutant synthetic internal or non-structural protein gene (or genes). Success was achieved by using: (i) a human influenza PB2 gene, that codes for a component of the viral polymerase, as the site for introducing attenuating mutation(s), and (ii) a selection system that favors the rescue of a synthetic PB2 gene into infectious virus. Selection was provided by using a single gene substitution reassortant virus that contained an avian influenza A PB2 gene on a background of wild type human influenza A genes as the helper virus for rescue of transfected human influenza A PB2 into infectious virus. The desired human influenza A mutant PB2 transfectant was favored in this situation because the avian influenza A PB2 gene restricts viral replication in mammalian cells, the substrate used for gene rescue in this instance, and hence viruses bearing the avian influenza gene were selected against. The feasibility of this approach was validated when we succeeded in: (i) introducing a coding mutation into a full length cDNA of a human influenza A PB2 gene, (ii) rescuing the RNA transcripts of the mutant cDNA into infectious virus by transfection and subsequent gene reassortment, and (iii) demonstrating that the resulting transfectant virus bearing the mutant gene exhibited the temperature-sensitivity and attenuation phenotypes predicted for this mutation. As a consequence, we are now optimistic that a genetically engineered PB2 gene which specifies a satisfactory level of attenuation can be rescued into a vaccine donor virus. This donor virus would be used in turn to transfer the mutant gene into newly emerged influenza A virus antigenic variants by gene reassortment. In this manner, satisfactorily attenuated and immunogenic live virus vaccine could be constructed rapidly to meet the threat of a new epidemic strain of influenza A virus.

Clinical Evaluation of Cold-Adapted (*ca*) Influenza A Virus Vaccine. A live attenuated influenza A cold-adapted (*ca*) candidate vaccine ($10^{6.0}$ $TCID_{50}$/vaccinee) was observed to be safe in infants less than six months of age. However, the immunogenicity of the vaccine was low in two-month-old infants with less than 35% responding to vaccine, whereas 90% of vaccinees who were 6 to 36 months old developed virus-specific antibodies. Maternally-derived antibodies appeared to suppress the immunogenicity of the vaccine in two-month-old infants. These findings suggest that it may be necessary to use a higher dose of vaccine (*e.g.*, $10^{7.0}$ $TCID_{50}$/vaccinee) in this age group to overcome the suppressive effect of maternal antibodies.

Clinical Evaluation of Candidate Live PIV3 Vaccines. The bovine parainfluenza virus type 3 (PIV3) is currently being evaluated as a "Jennerian" vaccine for prevention of disease caused by human PIV3. At a dose of $10^{5.0}$ $TCID_{50}$, the BPIV3 was safe and immunogenic in seronegative children. In addition, the attenuation phenotype of BPIV3 was stable during prolonged replication in humans. A dose of $10^{5.0}$ $TCID_{50}$ represents more than 100 human infectious dose 50's of the virus. These observations indicate that this candidate vaccine is satisfactorily infectious, safe, and immunogenic in seronegative humans. Additional studies involving BPIV3 are currently underway in humans. Another candidate vaccine virus, the above mentioned JS HPIV3 *cp*45, is also being evaluated in seronegative infants.

SIMIAN IMMUNODEFICIENCY VIRUSES (SIV)
(IMMUNODEFICIENCY VIRUSES SECTION)

Experimental Immunoprophylaxis. A crucial element in the development of effective therapeutic and prophylactic strategies for AIDS is an experimental animal that develops disease similar to human AIDS following infection. SIV infection of macaques is such a relevant model since this virus induces an immunodeficiency syndrome in infected macaques that is remarkably similar to human AIDS. In addition, SIV utilizes the CD4 molecule as a receptor, and the pathogenesis of disease appears similar to human AIDS. .Therefore, candidate vaccines can be evaluated not only for their ability to prevent infection but also for their ability to prevent AIDS.

A number of different strategies for inducing an SIV-specific immune response were investigated. An initial strategy that utilized whole inactivated SIV (WI-SIV) derived from a SIV proviral clone was highly successful. The vaccine prevented infection by an homologous or heterologous cell-free SIV challenge. However, this protection appears to have been mediated by antibodies generated to human cell proteins present in tight association with SIV as a consequence of its propagation in a human cell line during vaccine production. The basis for protection of monkeys with WI-SIV was analyzed by immunizing eight animals with WI-SIV and subsequently challenging these animals with either virus grown in human cells (SIV-hu) or macaque peripheral blood mononuclear cells, *i.e.*, PBMC (SIV-mac). Each of the animals challenged with SIV-hu was resistant to infection, whereas all animals challenged with SIV-mac became infected. A similar group of five immunized macaques were also challenged with PBMC-associated SIV and, as predicted from the previous experiment, each monkey became infected. However, long-term evaluation revealed that the immunized animals survived longer than the naive controls suggesting that immunization modified the course of disease. In this instance, the beneficial effect of immunization appeared to be virus-specific and could not be ascribed to human cell proteins.

A more quantitative neutralization assay for SIV was established by developing an adherent macaque CD4+ cell line. This cell line is susceptible to a wide range of SIV isolates, and virus infectivity and neutralizing antibody titers can now be determined by the enumeration of infected cells that are detected by immunoperoxidase labeling with SIV-specific antiserum.

New initiatives include the construction of: (i) vaccinia virus recombinants that express the SIV gag/pol and env (in collaboration with Dr. B. Moss and Dr. T. Fuerst), and (ii) integrase deletion mutants of SIV. These recombinants and SIV mutants were analyzed for expression of SIV proteins. In transient transfection assays, a vaccinia virus recombinant expressing envelope from a synthetic promoter was shown to produce 10- to 20-fold more protein than constructs under the control of the previously used 7.5K promoter. Vaccine trials utilizing these vaccinia virus recombinants are in progress. In addition, genetic immunization (*i.e.*, immunization with naked DNA that encodes protective antigens of SIV) is being evaluated.

SIV Pathogenesis. A crucial element in the development of effective therapeutic and prophylactic strategies for AIDS is an experimental animal in which infection produces disease similar in pathogenesis to the human disease. SIV infection of macaques is therefore a relevant model since SIV induces an immunodeficiency syndrome in infected macaques that is remarkably similar to human AIDS. An important use of this animal model system is the detailed study of pathogenesis and viral determinants of disease since many of these studies are not feasible in humans. These studies should aid us in determining how these primate lentiviruses destroy the immune system of their host, and this understanding will allow us to develop more rational therapeutic antiviral strategies.

In collaboration with the Yerkes Regional Primate Center, we studied the SIVsmm/PBj variant that produces fulminant, fatal disease within one to two weeks. Two of six full-length cDNA clones that were generated induced the acute lethal syndrome in inoculated pig-tailed macaques. Defined regions of the genome were exchanged between an acutely lethal SIVsmm/PBj clone and the related SIVsm clone, SIVsmH4, that produces the more typical AIDS syndrome. The viral envelope glycoprotein of the SIVsmm/PBj mutant was identified as one of the sites required for induction of the acute disease. However, other mutant genes were also required for expression of full virulence, namely gag and one of the regulatory genes. The virulence phenotype of chimeras correlated with mitogenic activity of virus for resting macaque PBMC *in vitro*.

Pathogenesis studies were expanded to include an analysis of SIVsm clones derived from an immunodeficient macaque in an attempt to identify viral factors contributing to the more traditional AIDS syndrome. This study of SIVsm clones derived from the spleen of an immunodeficient SIV-infected pig-tailed macaque identified a region analogous to the V3 loop of the HIV-1 envelope as responsible for cell tropism. Naturally-occurring variation in this region determined whether a virus infected macaque lymphocytes and/or macrophages and this tropism appeared to be linked to pathogenicity.

Early virologic events in SIV pathogenesis were studied in four macaques inoculated with SIVsm/E543 PBMC (derived from peripheral blood mononuclear cells of an infected monkey). Molecular analysis of viral DNA in PBMC demonstrated that each macaque was infected with a distinct variant. Variation in the envelope of virus present in PBMC, plasma, CSF, and lymph nodes samples is currently being evaluated. In collaboration with Dr. Lawrence Fox, LIR, samples from the four macaques were evaluated for elevation of cytokines during the early stage of infection. Although cytokines were not detected in plasma, PBMC cultured in the absence of mitogens, secreted TNF-alpha during a short period immediately prior to peak viremia. On the basis of this study, a trial of the cytokine-inhibitor, pentoxyfilline, during the acute-phase of infection is planned.

SIVagm is generally considered to be minimally pathogenic both for its natural host (African green monkey) and macaques. However, earlier, we observed that a SIVagm strain isolated from a captive vervet monkey (Agm9063) induced a persistent decline in CD4 lymphocytes in two pig-tailed macaques resulting in immunodeficiency and death of one of these macaques due to an *M. avium* infection. Virus (SIVagm9063) isolated from the spleen of this animal induced severe immunodeficiency in pig-tailed macaques resulting in death of three animals within 11 months of inoculation. Rhesus macaques and African green monkeys, in contrast, became persistently infected but did not develop evidence of immunodeficiency. Two infectious molecular clones were derived from a bacteriophage lambda library and both clones were shown to infect and replicate efficiently in pig-tailed macaque PBMC and macrophages. In addition, both clones were infectious for pig-tailed macaques. The sequence of one of the clones is being determined; initial analysis indicates that SIVagm9063 groups genetically with other vervet isolates but is distinct (80% identity in *gag* and *pol*).

HEPATITIS VIRUSES SECTION

FY 1993 was a banner year for the Hepatitis Viruses Section. An inactivated hepatitis A virus vaccine developed in collaboration with Dr. Ian Gust (Australia) and SmithKline-Beecham (CRADA AID 0004) was licensed in Europe during 1992 and should be licensed soon in the United States.

Search for New Hepatitis Viruses. Patients who undergo frequent transfusion, such as individuals with thalassemia in Mediterranean countries, often have evidence of chronic hepatitis. This is usually caused by hepatitis B virus (HBV) (with or without associated hepatitis D virus [HDV] or hepatitis C virus [HCV]). While most of the cases can be identified as chronic hepatitis B or hepatitis C, a number lack all serological markers for such infection, including antibody to multiple HCV antigens (second generation assays) and HBV or HCV sequences (as detected by PCR). The etiology of this form of chronic hepatitis (in some cases quite severe) remains unclear. These patients are being studied further and specimens from the most promising candidates were inoculated into chimpanzees in an attempt to identify and biologically amplify new hepatitis agents.

Hepatitis A Virus. Hepatitis A virus (HAV) is a picornavirus with a single-stranded RNA genome of approximately 7,500 nucleotides. The wild-type strain of HAV grows poorly in cell-culture, generally is not cytopathic, and yields a low titer of virus. A cell-culture adapted mutant has been isolated which grows significantly more efficiently in cell-culture and has the very interesting property of being attenuated for marmosets and chimpanzees.

Full-length infectious cDNA clones of a prototype virulent human HAV strain (HM-175) and its cell culture-adapted and attenuated derivative, HAV/7, have been generated and characterized in multiple cell-culture systems. Each clone has the characteristics of the virus from which it was derived. The two clones differ by 23 nucleotide changes that are scattered throughout the genome but are present somewhat more frequently in the 5' non-coding and P2 regions. Efficient growth in cell culture is dependent upon mutations in the 2B and 2C genes located within the P2 region of the HAV genome. Host cell specificity is altered by changes in the 5' non-coding region. Mutations located in either the 5' or 3' third of the genome markedly enhanced growth *in vitro* but only when they were combined with mutations within either the 2B or 2C gene of the P2 region.

Preliminary assessment of the mutations responsible for attenuation of HAV, strain HM-175, for two primate species (marmosets and chimpanzees) is now complete. Fourteen infectious chimeric constructs of HAV were prepared. These chimeras contained various combinations of sequences derived from the infectious cDNAs of wild-type virus and its attenuated derivative. Virulence of the chimeras for marmosets was compared to that of the wild-type virus and its fully attenuated mutant virus. There was a strong indication from these studies that virulence of the HM-175 strain of HAV is encoded in the P2 region of the genome. There is additional evidence that the genetic basis of virulence can be localized to the 2C gene of the P2 region. The 5' noncoding region of the virus does not appear to play a significant role in virulence but another domain, possibly in the P3 region of the genome, appears to play an interactive role with 2C in expression of virulence. In contrast to its role in viral replication in cell culture, gene 2B does not appear to be involved in virulence. Therefore, it may be possible to dissociate the phenotypes of attenuation and adaptation to cell culture.

Although the sites of growth-enhancing viral mutations have been mapped, the mechanisms by which these mutations affect virus growth *in vitro* remain to be identified. For example, information concerning functions that are up-regulated for tissue culture adapted mutants is needed to gain a more thorough understanding of the molecular aspects of HAV replication and to permit more rational design of mutants with desirable phenotypes.

Hepatitis E. Major epidemics of enterically transmitted non-A, non-B hepatitis (hepatitis E) have been reported in Asia, Africa, and North America. Similar cases of sporadic hepatitis, presumed to be hepatitis E, account for up to 90% of reported hepatitis in countries where hepatitis E is endemic. Hepatitis E virus (HEV) has been implicated in fulminant hepatitis of pregnancy, a disease with a 20% fatality rate. On the basis of electron microscopy and molecular characterization it appears that HEV belongs to the calicivirus family.

Various species of nonhuman primates were evaluated for their usefulness in the study of experimental HEV infection and disease. Significant differences in susceptibility were observed. In general, Old World species appeared to be more susceptible to infection with the Sar-55 strain of HEV than New World species and cynomolgus and rhesus macaques appeared to be the most useful experimental animal models.

Sequence analysis is now complete for the entire genome of two HEV strains: Sar-55 that was recovered from an HEV epidemic in Pakistan and Kashi-2 that was recovered from an epidemic of hepatitis E in China. A full-length cDNA of the Sar-55 strain was assembled from PCR-amplified sequences but initial attempts to demonstrate infectivity of this construct in cynomolgus monkeys by *in vivo* hepatic transfection were unsuccessful.

The genome of HEV encodes three open reading frames (ORFs). Open reading frame 1 is believed to encode the nonstructural proteins of the virus, whereas ORF-2 is thought to encode the capsid protein. The function of the gene product of ORF-3 is not known. The entire ORF-2 was expressed by a baculovirus recombinant and the resulting HEV protein was observed to be highly reactive with antibodies present in convalescent sera from patients and experimentally infected primates. Some of the viral protein expressed by the recombinant baculovirus spontaneously formed virus-like particulate structures that were reactive with HEV antibodies when studied by immune electron microscopy. This particulate antigen was compared with other expressed HEV antigens for its usefulness in diagnosis of HEV infection. It was found to be more sensitive for detecting IgM and IgG class anti-HEV than other antigens currently available. The particulate antigen expressed by the recombinant baculovirus is currently being evaluated in primates for its usefulness in active immunoprophylaxis.

Hepatitis C Virus. Currently, hepatitis C virus (HCV) appears to be the major etiological agent of transfusion-related non-A, non-B hepatitis. The HCV genome is a linear, positive-stranded RNA molecule of approximately 9,500 nucleotides and encodes a polyprotein of about 3,000 amino acids. Several stretches of amino acids in the HCV polyprotein share significant homology with flavivirus and pestivirus proteins, indicating that HCV is distantly related to these virus groups.

The recent cloning and sequencing of the genome of HCV led to the development of specific serologic tests for antibody to HCV that utilized recombinant-derived viral antigens. These tests have been useful for diagnostic and screening purposes but they shed little light on immunity to HCV, in part because currently available antigens are expressed from parts of the HCV genome that encode nonstructural or internal structural proteins. Furthermore, the finding of considerable genetic heterogeneity of the HCV genome, especially involving the genes encoding the envelope proteins, suggests that heterogeneity of viral envelope proteins may be as extensive as that observed for HIV. Such a finding does not bode well for attempts at vaccine development.

A sensitive and specific cDNA polymerase chain reaction assay was used to detect HCV RNA in serum samples from 128 unselected Southern African blacks with hepatocellular carcinoma (HCC). A significant number of these patients had evidence of current HCV infection, but not current HBV infection, suggesting that infection with HCV plays a role in the development of HCC in this population. Other major findings included: (i) identification of seven new genetic types of HCV, and (ii) biophysical characterization of light and heavy forms of HCV.

Preliminary evidence was obtained indicating that HCV can replicate, at least partially, in standard cell cultures *in vitro*. In collaboration with other scientists who were formerly fellows in LID, efforts are underway to extend these observations and to establish a useful *in vitro* system for the propagation of HCV that might prove useful for assay of neutralizing antibodies. Such an assay would diminish the need for chimpanzees in the evaluation of immunity to HCV. Recently, cell lines that are more permissive for HCV replication have been identified. These cells were then used to compare the infectivity of HCV *in vitro* with the infectivity titer determined previously in chimpanzees. Preliminary data confirm the sensitivity of the *in vitro* cultivation system and demonstrate a correlation between the infectivity titer of several HCV strains obtained *in vitro* and *in vivo*.

Hepatitis B Virus. Hepatitis B virus (HBV) is the prototype of the hepadnavirus family, which includes hepatotropic viruses that infect at least six separate host species. Infection with HBV causes polymorphic liver disease including acute or fulminant hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Over 300 million people throughout the world are chronically infected with HBV and are at high risk of developing hepatocellular carcinoma, one of the world's most prevalent forms of cancer.

The major surface (S) protein of the HBV envelope contains an antigenic determinant within a hydrophilic domain, designated the *a* determinant, that is believed to contain an epitope that induces and reacts with neutralizing or protective antibodies. Two major areas of study were pursued this year in an attempt to evaluate the importance of this epitope in virus infection. First, the protective efficacy of a human monoclonal antibody directed against the *a* determinant of the S protein was investigated in a seronegative chimpanzee. A single large dose of this monoclonal antibody was administered intravenously followed by challenge with wild-type HBV. The passively acquired antibody could be detected in serum for 40 weeks during which time there was no evidence of HBV replication. The chimpanzee subsequently developed acute hepatitis B approximately 1 year after challenge. Thus, passive prophylaxis with a monoclonal antibody directed against a putative major neutralization epitope of the HBV S protein significantly delayed but did not ultimately protect against infection.

Second, a series of putative neutralization escape mutants of HBV that sustained an amino acid substitution within the *a* determinant were studied in greater detail. These mutants were recovered from a group of individuals who had been vaccinated against HBV previously. Each viral isolate sustained an identical nucleotide substitution that resulted in a single amino acid change in the *a* determinant. Because the emergence of this mutant HBV raised questions about the efficacy of the existing recombinant vaccines for hepatitis B, two licensed vaccines were investigated for their ability to protect chimpanzees against challenge with the S gene mutant. Both recombinant vaccines provided protection against challenge with the mutant suggesting that properly vaccinated individuals are not at risk of infection by the S gene mutant virus.

Animal Hepadnaviruses. The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to HBV. Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. Thus, WHV infection of woodchucks provides a relevant and convenient experimental model for understanding HBV infection of humans.

All mammalian hepadnaviruses possess a gene, designated X, that encodes a protein capable of transactivating virus gene expression. The available evidence indicates that the X protein plays an important role in the process of oncogenic transformation of cells. However, research performed in other laboratories indicated that the HBV X protein was not essential for virus replication in transformed liver cell lines, but these studies did not address the possible need for this protein *in vivo*. In an effort to resolve this issue, oligonucleotide directed mutagenesis was used to introduce single nucleotide substitutions in the WHV X gene. Special care was taken to avoid changes in the overlapping genes and *cis*-acting elements in this complex region of the virus genome. Three amino acids could be removed from the carboxyl terminus of the X protein without abolishing the ability of the virus to replicate *in vivo* in woodchucks following direct transfection of the viral genome into the liver. In contrast, truncation of the carboxyl terminus of the X protein by 9 or more amino acids ablated *in vivo* infectivity. In addition, a mutant lacking the X protein initiation codon was not able to infect woodchucks. Furthermore, mutations that truncated the X protein at the amino terminus or altered key amino acids in the central domain of the protein also ablated *in vivo* infectivity. Altogether, 9 of 10 WHV X gene mutants were unable to replicate *in vivo*. These observations suggest that the hepadnavirus X gene is essential for virus replication *in vivo* and that the protein is highly sensitive to alteration.

In addition, characterization of the X gene promoter was extended. The location of the promoter was mapped and the essential nucleotides in this *cis*-acting element were identified. Two separate promoters were mapped within the WHV pregenome promoter. One promoter is located on the minus strand DNA, while the other is present on the plus strand DNA, indicating that this WHV promoter is bi-directional.

Finally, the complete nucleotide sequence of the New York strain of WHV was determined. In another related investigation, the nucleotide sequence of selected genomic regions was determined for over 100 independent clones derived at one time point from a chronically infected feral woodchuck. This was done in an attempt to ascertain the extent of sequence variation that occurred among WHV genomes during persistent infection of a wild-caught animal. The resulting sequence analysis (~70,000 nucleotides) yielded an estimated sequence divergence of 1.6 nucleotide differences per 10,000 nucleotides analyzed. Overall, significant progress has been made in the characterization of WHV gene products and *cis*-acting elements which must be understood in order to understand the mechanism of oncogenesis of this virus.

DENGUE VIRUSES (MOLECULAR VIRAL BIOLOGY SECTION)

The four serotypes of the dengue virus form a distinct antigenic subgroup within the 70 members of the arthropod-borne, serologically related flaviviruses. These flaviviruses are now classified within the flavivirus genus of the Flaviviridae which contains two other genera, *i.e.*, the genus for the human hepatitis C virus genus and genus for the pestiviruses. Dengue viruses continue to cause major epidemics in most tropical and subtropical regions where the *Aedes* mosquito vectors are abundant. Infection by dengue virus usually causes fever, rash, and joint pain, which are sometimes severe, but the disease is self-limited. However, in recent years, a more severe form of dengue, characterized by fever and hemorrhagic shock, has occurred with an increasing frequency, most notably in the dengue epidemic region of southeast Asia. This form of dengue has a high fatality rate, especially in children and young adults. A safe and effective vaccine against dengue is not available at this time.

Genetic Basis of Mouse Neurovirulence. The mouse-adapted mutant of dengue type 4 (DEN4) virus, strain H241, is highly neurovirulent for mice, whereas its non-mouse adapted parent is rarely neurovirulent. The genetic basis for neurovirulence of the mouse-adapted mutant was studied by comparing intratypic chimeric viruses that contained the three structural protein genes of the parental virus or the neurovirulent mutant on a background sequence of non-neurovirulent DEN4 virus, strain 814669. The chimera that contained the genes for the three structural proteins (C-preM-E) of mouse neurovirulent DEN4 virus, strain H241, proved to be highly neurovirulent in mice, whereas the chimera that contained the corresponding genes of its non-mouse adapted parent was not neurovirulent. These observations indicate that most of the genetic loci for neurovirulence of the DEN4 virus mutant map within the structural protein genes. A comparison of the amino acid sequences of the parental virus and its mouse neurovirulent mutant revealed that there were only five amino acid differences in the structural protein region and three of these were located in the envelope glycoprotein (E). Analysis of chimeras which were constructed to contain one or two of the variant amino acids of the mutant E sequence substituting for the corresponding sequence of the parental virus, identified two of these amino acid changes as important determinants of mouse neurovirulence. First, the single substitution of Ile for Thr_{155} which ablated one of the two conserved glycosylation sites in dengue E yielded a virus that was almost as neurovirulent as the mouse-adapted mutant. Thus, the loss of an E glycosylation site appears to play a major role in DEN4 neurovirulence. Second, the Leu for Phe_{401} substitution also yielded a neurovirulent virus but it was less neurovirulent than the glycosylation mutant.

The genetic basis for neurovirulence of a mouse-adapted mutant of dengue type 2 (DEN2) virus, strain NGC, was also investigated by analysis of chimeric dengue viruses. The genes for two or all three structural proteins of the mouse-adapted, neurovirulent DEN2 virus mutant were substituted for the corresponding genes of the full-length infectious cDNA clone of its non-neurovirulent DEN4 parental virus. Chimeric viruses (DEN2 C-preM-E neurovirulent)/DEN4 and (DEN2 preM-E neurovirulent)/DEN4 were both neurovirulent for suckling mice, but did not produce disease in 6-week-old mice, whereas the DEN2 virus neurovirulent mutant was lethal for these mice at low dose. This indicated that in the case of DEN2 virus some, but not all of the genetic loci responsible for neurovirulence were located in the structural protein genes. Sequence analysis of the cloned structural protein genes (C-preM-E) of the non-neurovirulent parental DEN2 virus and its neurovirulent DEN2 mutant identified 7 nucleotide substitutions in the neurovirulent mutant that produced a coding change. Two of the changes were in the pre-M gene, while the remaining 5 changes were located in the envelope glycoprotein (E) gene. A series of viruses have been constructed in which one or more of these mutations were introduced into the parental preM-E sequence present in a (DEN2 parent)/DEN4 chimeric virus. The testing of these chimeras in mice has been initiated.

Mutational Analysis of the Dengue Virus Genome 3' Non-Coding Region. The positive strand DEN4 virus RNA genome contains 384 nucleotides in the 3' noncoding region (NCR). Sequence analysis indicated that the 3' NCR contains two types of conserved sequences, designated CS-1 and CS-2, that are also present in other mosquito-borne flaviviruses. The last 82 nucleotides in DEN4 3' NCR form a stable stem-and-loop secondary structure similar to that found in other flaviviruses. Recently, functional analysis of the 3' NCR was initiated by deletion mutagenesis. A series of deletions in the 3' NCR ranging from 30 to 202 nucleotides in length were engineered into the full-length infectious cDNA of DEN4 virus and RNA transcripts of the mutant DNA constructs were tested for infectivity by transfecting permissive tissue culture cells. Viable dengue virus was recovered from mutant DNA constructs containing a deletion in one or the other or both of the two CS-2 sequences. In contrast, the last 112 nucleotides, including the stem-and-loop structure at the 3' end, the preceding CS-1 sequence, and 1~7 nucleotides upstream of CS-1 were found to be essential for infectivity of dengue virus. Most of the viable deletion mutants constructed in this manner were stable and produced plaques of reduced size on mosquito C6/36 cells compared to wild type virus. Analysis of plaque formation in primate-derived LLC-MK_2 cells revealed that most deletion mutants also produced plaques that were reduced in size and slow to develop. Furthermore, most mutants grew slowly and attained a lower titer in cell culture compared to parental wild type virus. These observations indicate that most DEN4 virus deletion mutants were restricted in their replicative capacity. An exception was observed for deletion mutant (Δ3' 303-183) which produced small plaques on C6/36 cells but nonetheless grew to a high titer similar to that of parental wild type virus.

In a later study, the deletion mutant (Δ3' 303-183) and four growth-restricted deletion mutants were compared to the wild type parental virus with respect to infectivity and immunogenicity in rhesus monkeys. For reasons presently not understood, none of the deletion mutants or the wild type virus induced viremia in monkeys. Nevertheless, mutant (Δ3' 303-183) induced an antibody response, measured by radioimmunoprecipitation or virus neutralization *in vitro*, that was similar to that of wild type virus. In contrast, the other DEN4 virus 3' NCR deletion mutants induced low to moderate levels of virus-specific antibody. The immunogenicity of these 3' deletion mutants in monkeys appeared to be directly related to the level of growth attained in infected simian LLC-MK_2 tissue culture cells. These observations suggest that all but one of the 3' deletion mutants are attenuated. These attenuated mutants should be evaluated in humans as candidate live virus vaccine strains.

Dengue Virus Subunit Protein Immunoprophylaxis. Earlier studies of vaccinia virus recombinants that expressed DEN4 virus E indicated that a C-terminally truncated E, approximately 80% in length, was more immunogenic and protective in mice than full-length E.

The increase of immunogenicity and protective efficacy associated with the specific truncation of E was initially thought to have implications for the development of effective subunit vaccines for dengue viruses as well as other flavivirus diseases. For this reason, recombinant baculoviruses were constructed that expressed 80%E of dengue virus as well as other flaviviruses. The baculovirus-expressed 80%E proteins of DEN4 or DEN2 virus were secreted into the fluid medium of infected insect sf9 cells and were stable. The relative immunogenicity of the intracellular products of b(DEN4, 80%E) and b(DEN4, 93%E) as well as secreted 80%E concentrated from tissue culture medium was determined by inoculating mice with the same amounts of these materials and assessing subsequent antibody response. Each of the mice inoculated with secreted 80%E developed antibodies to the E protein, whereas mice inoculated with a lysate of cells infected with b(DEN4, 93%E) or b(DEN4, 80%E) developed little if any E-specific antibody as determined by radioimmunoprecipitation. Based on these encouraging observations with secreted 80%E, baculovirus-expressed DEN4 virus 80%E was evaluated in monkeys to determine if its immunogenicity had been enhanced for primates. Groups of rhesus monkeys were inoculated three times intramuscularly with: (i) a lysate of cells infected with b(DEN4, 80%E), (ii) concentrated fluid medium from the cultures of these cells, (iii) a lysate of cells infected with b(DEN4, 93%E), (iv) a lysate of cells infected with wild type baculovirus, or (v) concentrated medium from a culture of wild type baculovirus infected-cells. The antibody response to immunization as determined by plaque reduction neutralization was low in each group inoculated with DEN4 virus material. Subsequently, the monkeys were challenged with DEN4 virus and tested for development of viremia using mosquito C6/36 cells. The results of these tests could not be interpreted in terms of protective effect of immunization because unimmunized control monkeys did not regularly become viremic. Although the protective efficacy of the recombinant baculovirus products could not be evaluated in monkeys, it was evident that these materials were at best, weakly immunogenic.

Tick-Borne Encephalitis Virus, A Related Flavivirus. Studies involving Russian spring-summer encephalitis (RSSE) virus represent a logical extension of ongoing studies of the dengue viruses. RSSE virus and its close relatives constitute the tick-borne encephalitis (TBE) subgroup of the flaviviruses. RSSE and its TBE relatives cause serious disease with high mortality throughout Russia, India, Scandinavia and Central Europe. In addition, a virulent member of the TBE subgroup, Powassan virus, is present in Canada and the Eastern and Western US. Twenty cases of Powassan encephalitis have been reported in the US and Canada but more ominous is the wide distribution of the virus in small mammals.

Following our success in constructing full-length DEN4 virus cDNA that can be transcribed to produce infectious RNA, efforts were initiated to employ this cDNA to produce infectious chimeric DEN4/TBE viruses that could be used to: (i) identify the genetic determinants of TBEV neurovirulence, and (ii) develop attenuated candidate live virus vaccine strains for prevention of tick-borne encephalitis. Chimeric TBE/DEN4 cDNAs were prepared and RNA transcripts of these cDNAs were tested for infectivity in permissive simian cells in culture. Two new viable chimeric flaviviruses were recovered. These chimeric viruses contained TBEV C-preM-E or preM-E structural protein genes with the remaining genes and noncoding sequences derived from DEN4 virus. Evaluation of the chimeric TBE (preM-E)/DEN4 virus in mice indicated that it caused fatal encephalitis in suckling or adult mice following intracerebral inoculation, whereas adult mice inoculated with DEN4 virus remained free of disease and very few suckling mice developed encephalitis. Thus, the chimeric virus retained the neurovirulence of its TBEV parent from which its preM and E genes were derived. In contrast, unlike parental TBEV, chimeric TBE (preM-E)/DEN4 virus was not virulent when adult BALB/c mice were inoculated peripherally, indicating a loss of neuroinvasiveness. Of note was the observation that mice inoculated peripherally with TBE (preM-E)/DEN4 virus were protected against subsequent intraperitoneal challenge with a lethal dose of TBEV.

Success in constructing a viable TBE/DEN4 virus chimera that retained the protective antigens of TBEV but lacked the peripheral invasiveness of TBEV provides the basis for pursuing the development of an attenuated TBEV vaccine. However, before this goal can be realized, additional modifications of the chimera must be achieved in order to ablate neurovirulence for the CNS that is manifest following direct inoculation of virus into the brain. For this reason, the preM-E chimeric virus was subjected to mutational analysis in an attempt to reduce or ablate neurovirulence that is evident when virus is inoculated directly into the brain. Three distinct mutations that were independently associated with a significant reduction of mouse neurovirulence of the preM-E chimera were identified. These mutations ablated: (i) the TBEV PreM cleavage site which is required for proper processing of M protein; (ii) the TBEV E (envelope glycoprotein) glycosylation site; or (iii) the first DEN4 virus NS1 (non-structural protein one) glycosylation site. In contrast to the down-regulation of neurovirulence by the preceding mutations, ablation of the second DEN4 virus NS1 glycosylation site or the TBE PreM glycosylation site or amino acid substitution at two positions in the TBEV E protein effected a moderate increase in neurovirulence. The only conserved feature of the 3 attenuated mutants was restriction of virus growth yield in both simian and mosquito cells in culture. Following parenteral inoculation, these attenuated mutants induced complete resistance in mice to fatal encephalitis caused by subsequent challenge with the highly neurovirulent preM-E chimera. Our success in ablating neuroinvasiveness and neurovirulence that is manifest when TBEV is inoculated directly into the brain, suggests that the strategy used to achieve these effects might yield a safe, effective live attenuated TBEV vaccine.

EPIDEMIOLOGY SECTION

Rotavirus Molecular Biology and Initial Attempts to Rescue Rotavirus Genes Encoded by Viral cDNA. Initial efforts to rescue rotavirus genes encoded by cDNA have produced encouraging results. Recent studies have demonstrated that biologic activity can be achieved using a synthetic rotavirus RNA analog made *in vitro* from cDNA that also incorporates the coding region of a heterologous reporter gene. This system represents a powerful tool for defining the *cis*-acting nucleotide sequences and *trans*-acting viral proteins involved in rotavirus replication. It may also represent the first step in the development of a technique for introducing synthetic rotavirus RNA genes into infectious rotavirus.

cDNAs were constructed to encode plus- or minus-sense analogs of porcine rotavirus gene 9 RNA that encodes outer capsid protein VP7. The VP7 coding sequence was deleted and replaced with the coding sequence of the CAT gene which was thus flanked by the non-coding 5'-terminal 44 nucleotides and the non-coding 3'-terminal 35 nucleotides of the authentic rotavirus gene. Linearization of cDNA with *HgaI* and transcription *in vitro* by T7 RNA polymerase produced gene 9-CAT RNAs which contained the exact, correct gene 9 5' and 3' ends. Transfection of plus-sense gene 9-CAT RNA into rotavirus-infected cells resulted in the efficient expression of CAT; this was greatly enhanced by the presence of a 5' cap structure. Expression of CAT was ablated by omitting rotavirus superinfection or by removing the 3'-terminal 35 nucleotide rotavirus sequence from the RNA. This indicated that amplification was dependent both on rotavirus proteins supplied *in trans* and on *cis*-acting 3' terminal rotavirus sequences. Deletion analysis indicated that only the the 3' 19 terminal nucleotides were required for efficient amplification and expression of the synthetic gene. Minus-sense or double-stranded gene 9-CAT RNA was essentially inactive, indicating that synthetic RNAs can be introduced into the rotavirus replicative cycle in cells only when provided in the plus sense. Packaging of the CAT-bearing chimeric RNA into rotavirus particles did occur with low efficiency.

The effect of substituting rotavirus non-coding sequences from a different genome segment was also tested; for this purpose a second analog was constructed that contained the CAT gene flanked by the non-coding regions of gene 11 of the simian rotavirus SA-11. In addition, in order to determine whether the viral orfs which had been replaced by the CAT orf might contain *cis*-acting signals, a third analog contained porcine rotavirus gene 9 in its entirety with the CAT orf inserted following the end of the 5' non-coding region, *i.e.*, nucleotide position 44. Transfection of these analogs individually into human 293 cells infected with porcine rotavirus (strain OSU) resulted in the efficient expression of CAT. Furthermore, each of the three CAT-containing RNA analogs was packaged into a form that could transmit CAT expression to fresh cells for at least two serial passages. Passage of CAT expression was insensitive to ribonuclease but was neutralized by guinea pig antiserum raised against porcine rotavirus, suggesting that the analog was packaged within virus particles. The amount of CAT expression following passage was much less than that observed during the initial transfection. The three different RNA analogs were essentially indistinguishable in their efficiency of CAT expression and passage. This supports the view that the *cis*-acting sequences required for all aspects of the rotavirus replicative cycle are located in the rotavirus non-coding regions and are not augmented by the inclusion of additional viral sequences.

Molecular Epidemiology of Rotaviruses. The molecular epidemiology of human and animal rotaviruses was investigated employing hybridization and sequencing techniques with the objective of expanding our understanding of the role of the viral outer capsid protein VP4 in virulence of human rotaviruses. Previous studies in LID suggested that the fourth viral gene (*i.e.*, the VP4 gene) is a major determinant of the reduced virulence of rotaviruses that produce asymptomatic infection in newborn nurseries. As a consequence of their reduced virulence these viruses are able to maintain transmission in the nursery over a period of years because infection is usually not detectable clinically. This concept that VP4 was responsible for reduced virulence was based on the high degree of nucleotide sequence homology of the fourth gene of the M37 strain, (a VP7 serotype 1 virus originally isolated from an asymptomatic newborn in Venezuela) with the corresponding gene of other "nursery" strains that belonged to other VP7 serotypes and that were responsible for similar asymptomatic infections in England, Australia and Sweden. Moreover, the conserved "nursery" strain VP4 was quite distinct from the VP4 of rotavirus strains isolated from older infants with diarrhea. Studies were performed to confirm and expand these observations. Other similar "nursery" strains were examined,including viruses recovered from additional newborn nurseries in Venezuela, South Africa, Australia, the United States and Chile. Rotavirus strains recovered from older infants and young children with diarrhea were also evaluated for the purpose of comparison. Specific hybridization probes were developed to detect the "M37" VP4 allele, representative of "nursery" strains, and the Wa VP4 allele, representative of strains associated with illness in older children. The results of these studies confirmed our previous observation that the "M37" VP4 allele was associated, in most instances, with a high level of prolonged, silent transmission in the newborn nursery. This supported our previous hypothesis that the "M37" VP4 was responsible for the low virulence of nursery strains that persisted in the nursery over a long period of time. However, in one nursery in Venezuela and one in Germany, where "M37-like" strains persisted, the endemic strains were associated with some cases of diarrhea in newborns, mostly premature infants, but also full-term babies.

The finding that some of the ill newborns examined in Venezuela and Germany, and that some older infants and young children with diarrhea in Venezuela and South Africa shed viruses with the "M37" VP4 allele contradicts our previous observation that "M37"-like viruses are invariably completely attenuated. In addition, the finding that "Wa-like" strains, commonly associated with diarrhea in infants and young children, can induce asymptomatic infection in newborns suggests that other factors, such as maternally derived neutralizing antibodies, might be involved in modulating the clinical outcome of infection that occurs shortly after birth.

Rotavirus Pathogenesis. A study performed in newborn piglets led to the identification of the viral genes that are essential for virulence of porcine rotavirus in its natural host. The response of gnotobiotic newborn pigs to: (i) a virulent porcine rotavirus (strain SB-1A), (ii) a human rotavirus (strain DS-1) avirulent for pigs, or (iii) reassortants derived from these two viruses was studied intensively, both clinically and virologically. Our analysis of the data indicated that the 3rd (VP3), 4th (VP4), 9th (VP7), or 10th (NS28) porcine rotavirus gene each played an important independent role in virulence of rotavirus infection in piglets. Replacement of the 3rd, 4th, 9th or 10th gene of virulent porcine rotavirus SB1A with the corresponding gene of avirulent human rotavirus DS-1 ablated virulence of the reassortant for gnotobiotic piglets. Although each of these genes played an essential role in virulence, all four genes were required to convert the avirulent DS-1 rotavirus to a virus that caused disease in piglets. Thus, each of these porcine rotavirus genes must be present in order for porcine rotavirus to be fully virulent for its natural host.

Live Attenuated Rotavirus Vaccines. Rotaviruses have consistently been shown to be the single most important etiologic agents of severe diarrhea in infants and young children in both developed and developing countries. These viruses are responsible for 30-50% of such severe diarrheal disease. In the United States, rotavirus disease is estimated to be responsible for: (i) 110,000 hospital admissions annually for a total of 583,000 hospital days, and (ii) approximately 75-150 deaths. In the developing countries, however, rotaviruses are estimated to cause over 17 million cases of moderately severe or severe diarrhea and over 870,000 deaths annually. In both developed and developing countries there is an obvious need for a rotavirus vaccine that is effective in preventing severe rotavirus diarrhea during the first two years of life when this disease takes its greatest toll. We have chosen to use the "Jennerian" approach to vaccination, which involves the use of a live vaccine virus strain of non-human origin that is attenuated for humans. In our studies, the surrogate rotavirus strain was derived from a rhesus monkey, while others have used a bovine rotavirus for this purpose.

Immunization with a monovalent animal rotavirus has had limited success because serotype-specific immunity against each of the four clinically important human rotavirus VP7 serotypes could not be achieved consistently in infants less than six months of age. As a consequence, the "Jennerian" approach was expanded to provide the required broader antigenic coverage. This was achieved by formulating a quadrivalent rotavirus vaccine that contains: (i) rhesus rotavirus (RRV) (VP7 serotype 3), and (ii) three human rotavirus-RRV reassortants, each possessing 10 RRV genes and a single human rotavirus gene that encodes VP7 serotype 1, 2, or 4 specificity. The quadrivalent vaccine was shown to be safe and moderately antigenic at a dose of 10^5 plaque forming units, achieving in young infants a "take rate" of ~50% for each component. A total of 9,500 infants and young children in the United States or overseas have participated or are currently participating in placebo-controlled field trials of individual reassortant vaccines or the quadrivalent vaccine.

The modified strategy appears to be quite promising with regard to achieving our goal which is prevention of the serious consequences of rotavirus infection, namely severe diarrhea. A reduction in the incidence of all forms of rotavirus diarrhea was observed in most, but not all, of the placebo-controlled, double-blind clinical trials. However, the quadrivalent RRV vaccine has consistently proven to be very effective in preventing severe dehydrating rotavirus diarrhea which is the form of rotavirus disease that kills infants and young children. As a consequence, the prevention of severe dehydrating diarrhea has been adopted as the end point for vaccine efficacy in the large "catchment" trial currently underway in Venezuela as well as in other large trials currently being planned.

Alternative Strategies for Rotavirus Immunoprophylaxis. Rotaviruses possess two outer capsid proteins that independently induce neutralizing antibodies and resistance to virus infection. Of these two rotavirus protective antigens, VP4 and VP7, only clinically and epidemiologically relevant alleles of the latter are included in the quadrivalent rhesus rotavirus/rhesus rotavirus reassortant vaccine now undergoing extensive clinical evaluation. Each of the vaccine viruses has a VP4 of simian rotavirus origin. Unfortunately, this VP4 is not related antigenically to the VP4 present on most virulent human rotaviruses.

In case the quadrivalent rhesus rotavirus-based vaccine does not exhibit the desired protective efficacy, it may be necessary to increase the effectiveness of live rotavirus vaccine by including a rotavirus that contains a VP4 that is closely related to the VP4 of most virulent human rotaviruses. For this reason efforts were initiated to produce such a virus by cold-adaptation of several human rotaviruses. This approach has been successful in producing attenuated mutants of influenza A and B viruses, parainfluenza type 3 virus and respiratory syncytial virus. Thus, far, 4 human rotaviruses have been adapted to grow efficiently in cell culture at a low temperature (26°C) that is highly restrictive for growth of the parental virus. These 26°C viruses are indeed cold-adapted (*ca*) mutants because they produce plaques efficiently at 26°C, whereas the parental viruses do not. These candidate *ca* mutant vaccine strains are being prepared for clinical evaluation in humans.

Acute Non-Bacterial Gastroenteritis Caliciviruses. The 27 nm Norwalk virus was discovered by LID scientists more than 20 years ago, and was subsequently shown by us to be a major cause of outbreaks of acute nonbacterial gastroenteritis. Despite the clinical importance of Norwalk virus and Norwalk-like viruses, progress in elucidating the epidemiology and the molecular properties of these agents has been very slow. Limited progress can be ascribed to our failure to: (i) grow the agent in cell culture, or (ii) identify a satisfactory experimental animal model in which to study pathogenesis and immunity. Recently, the genome of Norwalk virus was cloned and sequenced (Jiang and Estes, Baylor College of Medicine). The genomic organization of the Norwalk virus was found to be similar to that of the caliciviruses, with the 5' end of the genome encoding the non-structural proteins and the 3' end encoding a single structural protein and an additional protein of unknown function.

Our sequence analysis of other small round viruses associated temporally with acute non-bacterial gastroenteritis indicated that many of these agents were related to the prototype Norwalk calicivirus, however, significant sequence divergence was observed suggesting that considerable polymorphism exists within this group of ubiquitous enteric viral pathogens.

Serological surveys for infection by Norwalk virus and related viruses were performed using baculovirus recombinant-expressed Norwalk virus capsid protein as antigen. Of particular interest were observations made during an investigation of a cohort of 240 Finnish infants and children who were maintained under surveillance over a two year interval during a rotavirus vaccine trial. Forty-nine percent of this population developed a serological response to Norwalk virus as measured by ELISA. In Finland, Norwalk virus (or "Norwalk-like" virus) infection appears to be common during early childhood, however, the infections detected by serological analysis could not be associated with a specific episode of gastroenteritis. Either attenuated strains of virus were active in the population or Norwalk virus infection exhibits an age effect similar to that observed for HAV in which disease is manifest mainly in older individuals.

HONORS AND AWARDS

Robert M. Chanock, M.D.

Invited speaker, Wellcome Symposium on Antiviral Therapy: Innovations in Therapy of Human Viral Diseases. December 6-9, 1992, Research Triangle Park, NC. Lecture: "New techniques for generation of human viral antibodies: application of such antibodies to prevention and treatment of viral diseases"

Co-organizer, Wallace P. Rowe Ninth Annual Symposium on Animal Virology, NIH, February 1-2, 1993

Invited participant, 1993 Frontiers in Biomedical Research Symposium, Annenberg Center at Eisenhower Medical Center, Rancho Mirage, CA, February 14-18, 1993. Sponsored by the Annenberg Center, Karolinska Institutet (Stockholm, Sweden), Memorial Sloan-Kettering Cancer Center (NY, NY) and The Scripps Research Institute (La Jolla, CA).

Invited speaker, Symposium in honor of Dr. Harold Ginsberg, held at Department of Microbiology, College of Physicians & Surgeons of Columbia University, (NY, NY) May 3, 1993. Title of Presentation: "Respiratory Syncytial Virus: New Opportunities to Control This Important Respiratory Tract Pathogen."

Invited speaker, Spring Animal Virology Seminar, Harvard Medical School, Boston, MA, March 11, 1993. Title of presentation: "Human viral antibodies: often unappreciated soldiers of the immune resistance."

Member, National Academy of Sciences Committee on International Security and Arms Control, Working Group on Biological Weapons Control. Participated in Bilateral Meeting with Vice President and Academicians of the Russian Academy of Sciences, Aspen Institute, Wye, MD May 27-29, 1993.

Co-organizer, Modern Approaches to New Vaccines Including the Prevention of AIDS Conference, Cold Spring Harbor Laboratory, Long Island, NY September 20-24, 1993.

Session Chairman, National Academy of Sciences Colloqium on Changes in Human Ecology and Behavior: Effects on Infectious Diseases. National Academy of Sciences, Washington, D.C. September 27-28, 1993.

Vanessa M. Hirsch, D.V.M., D.Sci.

Chairperson, Session on Molecular Biology of SIV and HIV, The 10th Annual Symposium on Nonhuman Primate Models for AIDS, San Juan, Puerto Rico, November 17-21, 1992.

Program Committee for forthcoming Conference on Simian AIDS, New England Regional Primate Research Center, February 8, 1993, "SIV Infection of Macaques."

Invited ad hoc member of the Regional Primate Research Center's Scientific Advisory Committee, Seattle, WA, May 12-15, 1993.

Invited to serve on Program Committee for Non-human Primate Models for AIDS Meeting, Madison, WS, June 24-26, 1993.

Albert Z. Kapikian, M.D.

Invited to be chairman (convenor) of session on Rotaviruses (2) on July 14, 1992, at Annual Meeting of the American Society for Virology at Cornell University, Ithaca, New York, July 11-15, 1992.

Invited speaker, 46th Annual Meeting of the Japanese Society for Bacteriology, September 3-4, 1992, Sendai, Japan. Made presentation entitled "Recent Advances in Development of Rotavirus Vaccine" on September 3, 1992.

Invited speaker, 26th Joint Conference on Viral Diseases of the Japan-United States Cooperative Medical Science Program, September 8-10, 1992, Nara, Japan. Made presentation entitled "Efficacy of the 'Jennerian' and Modified 'Jennerian' Approach to Vaccination of Infants and Young Children Against Rotavirus Diarrhea Using a Rhesus Rotavirus (RRV) and Human-RRV Reassortant Vaccine" on September 8, 1992.

Invited to be Chairman of Session on Virology I on September 17, 1993, at meeting, "Modern Approaches to New Vaccines Including Prevention of AIDS," Cold Spring Harbor, New York, September 16-20, 1992.

Invited to make presentation on rotavirus infections and vaccines at Infectious Disease Friday Clinical Center Care Management Conference at NIH on November 6, 1992.

Invited to make presentation on viral gastroenteritis at FAES Graduate School course on medical virology, NIH, December 2, 1992.

Invited speaker, Ninth Annual Clinical Virology Symposium and Annual Meeting of PAGRVD in Clearwater, FL, April 25-28, 1993, "Rotavirus vaccine: How soon a reality?"

Recipient of PAGRVD Diagnostic Virology Award sponsored by Murex Corp.

Invited speaker, NIAID International Centers for Tropical Disease Research, Bethesda, MD, April 28-30, 1993, "Recent advances in the development of a rotavirus vaccine for the prevention of severe rotavirus diarrhea in infants and young children."

Invited speaker at 104th Ross Conference, "Strategies for Pediatric Vaccines: Conventional and Molecular Approaches," San Diego, CA, September 19-22, 1993.

Ching-Juh Lai, Ph.D.

Invited speaker, XIII International Congress for Tropical Medicine and Malaria: 50th Anniversary of the Isolation of Dengue Virus, November 29 - December 4, 1992, Bangkok, Thailand. Title of lecture: "Recent progress in the development of a new vaccine strategy against dengue virus infection."

Invited speaker, A Workshop on the Pathogenesis of Dengue and Dengue Hemorrhagic Fever, December 5, 1992, Bangkok, Thailand. Title of lecture: "Determination of genetic loci responsible for dengue virus neurovirulence in mice."

Invited speaker, Seminar on Dengue viruses at SmithKline-Beechem, February 28 - March 3, 1993, Brussels, Belgium. "Development of a new vaccine strategy against dengue virus infections."

Invited speaker, New Jersey's University of the Health Sciences, Piscataway, NJ, May 20, 1993, "Molecular approaches to dengue and other flavivirus vaccines"

Brian R. Murphy, M.D.

Chairman, Session on Bacteriology and Parasitology at the Cold Spring Harbor Meeting on "Modern Approaches to New Vaccines Including Prevention of AIDS," September 16-20, 1992.

Chairman, Wyeth Ayerst-LID CRADA Comittee on the Development of Live Attenuated Respiratory Syncytial Virus Vaccines, 1992-Present.

Speaker at the NIH Clinical Center Grand Rounds on "Vaccines for Influenza," September 23, 1992.

Invited speaker, Johns Hopkins Pediatric Vaccine Seminar Series, November 1992.

Member, NIH Institutional Biosafety Committee, 1993-Present.

Invited speaker at meeting, "Protective and Disease Enhancing Immune Response to RSV," May 1993 sponsored by NIAID, CDC, and WHO. "Summary of Past Experiences with Formalin-Inactivated Respiratory Syncytial Virus Vaccine."

Workshop Co-chairman on Respiratory Viruses, IXth International Congress of Virology, Glasgow, Scotland, August 1993.

Invited speaker, 104th Ross Conference on Pediatric Research, September 18-21, 1993, San Diego, CA, "Strategies for pediatric vaccines: Conventional and molecular approaches"

Robert H. Purcell, M.D.

Invited speaker, The International Symposium on Viral Hepatitis and Liver Disease, Tokyo, Japan, May 11-15, 1993.

Invited speaker, Turkish Microbiological Society, Istanbul, Turkey, June 27 - July 3, 1993.

Chairman, Workshop on Hepatitis Viruses, The IXth International Congress for Virology, Glasgow, Scotland, August 8-13, 1993.

Peter L. Collins, Ph.D.

Member of the Paramyxovirus Study Section, International Committee for the Taxonomy of Viruses.

Supervisory Committee for the Ph.D. thesis of Kyeong H. Park, "Molecular analysis of the Sendai virus genome," Mount Sinai School of Medicine, 1992.

Invited speaker, University of Maryland, September 1992. "Genetics of negative strand RNA viruses: a method for the direct manipulation of viral genomic RNA"

Invited speaker and session chairman, "Modern Approaches to New Vaccines Including the Prevention of AIDS," September 1992. "Rescue of synthetic helper-dependent analogs of the genomic RNAs of respiratory syncytial virus and parainfluenza virus type 3" and "Expression of a synthetic rotavirus genomic RNA segment bearing a foreign marker gene."

Invited speaker, Twinbrook II, NIAID, December 1992. "Nonsegmented negative strand RNA viruses: mapping the genomic RNA signals for encapsidation, transcription and replication."

NIAID Grand Rounds, December 1992. "Under the hood and working on the engine for nonsegmented negative strand viruses."

Committee for New Initiatives, 1993.

NIAID Peptide Users Committee.

Invited speaker, International Meetings in Biology, Madrid, Spain, February 1-3, 1993, "Rescue of synthetic analogs of respriatory syncytial virus genomic RNA" and "Cis-acting signals in the human parainfluenza virus 3 genome."

Invited speaker, Cleveland Clinic and Case Western Medical School, February 24-26, 1993, "New molecular genetic approaches for negative strand RNA viruses."

Invited speaker, WHO-CDC-NIAID Conference on Respiratory Syncytial Virus, May 1993. "Live attenuated virus vaccines: update on progress towards producing infectious virus from cDNA."

Invited workshop chairman, International Congress for Virology, August 1993.

Editorial Board, *Journal of Virology* and *Journal of General Virology*.

Adjunct professorship, University of Maryland, College Park.

James E. Crowe, Jr., M.D.

Invited to speak at World Health Organization / National Institutes of Health / Centers for Disease Control Meeting, "The Protective and Disease Enhancing Immune Response to

RSV," May 26-27, 1993, Bethesda, MD. Presentation, "Live Respiratory Syncytial Virus Vaccines."

Suzanne U. Emerson, Ph.D.

Invited speaker, Third International Positive-Strand RNA Virus Conference, Clearwater, Florida, September 23, 1992. "*In vivo* transfection by hepatitis A virus synthetic RNA"

Member, Concept Review Committee for the American Type Culture Collection. April 1-2, 1993.

Serve on the New Initiatives Committee for NIAID. January 1993-present.

Received "NIAID Director's Award," May 1993.

Co-chairperson, Persistent Viral Infections Workshop, NIH Research Festival, September 22, 1993.

Mario Gorziglia, Ph.D.

Invited speaker, Fourth International Symposium on Double-Stranded RNA Viruses, Scottsdale, AR, December 12-16, 1992. "Mapping *cis*-acting Nucleotides in the 5'- and 3'-Noncoding Regions of Rotavirus Genomic RNA."

Invited speaker, Third International Course on Genetic Engineering, Caracas, Venezuela, September 6-24, 1993.

Kim Y. Green, Ph.D.

Membership into the American Society for Virology, August, 1992.

Invited to serve as a member of the Ph.D. committed of Blanca Lupiani at the University of Maryland, College Park, Maryland, September 1992.

Invited speaker, American Society of Virology, University of California, Davis, to present paper entitled "Sequence Analysis of Wild Type Rotavirus Strains Collected in Rochester, New York During Two Rhesus Rotavirus Vaccine Studies," July 1993.

Roger H. Miller, Ph.D.

Session chairman, Molecular Biology of Hepatitis B Virus Meeting, Washington, DC, August 1993.

Norio Ogata, M.D.

Invited speaker, VIII International Symposium on Viral Hepatitis and Liver Disease, May 8-18, 1993.

E. Kanta Subbarao, M.D.

Invited lecture, Principles of Virology Course "Influenza Viruses", Spring, 1993, Johns Hopkins University School of Public Health.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00323-12 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Parainfluenza Virus Type 3 (PIV3) Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter L. Collins, Ph.D.	Supvy. Microbiologist	LID, NIAID
Others:	Kenneth D. Dimock, Ph.D.	N.I.H. Special Volunteer	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An experimental system was developed for human parainfluenza virus type 3 (PIV3) that represents the first available method for the direct genetic manipulation of viral genomic RNA (vRNA) sequences. Specifically, cDNA was constructed to encode a truncated synthetic vRNA analog in which all of the viral genes have been removed and replaced by the bacterial chloramphenicol acetyl transferase (CAT) gene. The cDNA is under the control of the promoter for T7 RNA polymerase and, at the distal end, contains an Hga1 site (in the DNA template) or a self-cleaving ribozyme (in the RNA product) for linearization. Transcription of the cDNA *in vitro* yields a negative-sense RNA containing the exact, correct vRNA termini. The RNA was transfected into tissue culture cells which were infected with PIV3 to supply complementing proteins. Under these conditions, the RNA was rendered biologically active ("rescued") as evidenced by the intracellular expression of CAT and by the production of a component (presumably infectious particles) that was released into the medium and could be passaged productively onto fresh cells in the presence of helper virus. Together with a similar system developed by us for human respiratory syncytial virus (RSV) (accompanying report) and by others for Sendai virus, this represents the first experimental method by which one can synthesize, and thereby manipulate, biologically-active analogs of the vRNA of a nonsegmented negative strand virus. It will now be possible to directly identify and characterize *cis*-acting signals in vRNA and, as described here, considerable progress has been made in this area. It should also be possible to modify the system such that rescue is complemented by cDNA-encoded viral proteins in place of PIV3. This would make it possible to characterize the roles of the proteins in replication, transcription and virion morphogenesis. Importantly, the success in rescuing PIV3-CAT supports the idea that it will be possible to adapt the system such that a complete synthetic vRNA can be used to produce nondefective virus. This would make it possible to create viruses that contain defined mutations for vaccine and experimental purposes.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00324-12 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Studies of Influenza Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Kanta Subbarao, M.D.	Senior Staff Fellow	LID, NIAID
	Cassandra Lawson, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*
St. Jude Children's Hospital (Webster, Kawaoka); FDA (Epstein); NIAID, LVD (Bennink and Yewdell)

LAB/BRANCH
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SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	2.1	1.5

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
    ☐ (a1) Minors
    ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new strategy for the construction of attenuated influenza A viruses was developed and implemented. This new approach involved the introduction of one or more specific mutations into the cDNA of a viral gene by site-specific mutagenesis followed by the rescue of full-length RNA transcripts of the gene into infectious virus. This means that it is now possible to generate an attenuated virus with defined mutations and use such a virus as a donor of its attenuating gene (or genes) to produce live virus vaccine whenever a new antigenic variant emerges in nature and poses an epidemic threat. The technique for rescue of a synthetic RNA influenza virus gene originally developed several years ago by other scientists could not be employed because its use was limited to genes for viral surface glycoproteins which must be derived from the new epidemic strain. Success in our endeavor required that mutant genes coding for internal proteins of the virus be rescued so such genes could be transferred to new antigenic variants. This was achieved through the use of very strong selective pressure.

The feasibility of this approach was validated when we succeeded in: (i) introducing a coding mutation into the gene for the internal protein PB2, (ii) rescuing the RNA transcripts of the mutant cDNA into infectious virus by transfection and reassortment, and (iii) demonstrating that the resulting transfectant virus bearing the mutant gene acquired the temperature-sensitivity and attenuation phenotypes predicted for this mutation.

Gull influenza A virus NS and NP genes and the human influenza A virus H1 hemagglutinin and/or N1 neuraminidase genes appear incompatible. This indicated that our strategy for developing live attenuated gull-human influenza A reassortants as vaccines for humans was unlikely to succeed since attenuated influenza A H1N1 virus reassortants bearing the full set of six transferrable gull genes could not be isolated using selection techniques that had worked regularly in the past.

The major protective antigens of the influenza A virus were shown to be the hemagglutinin (HA) and neuraminidase (NA) proteins and the immunity induced by these antigens was mediated solely by antibodies. $CD8^+$ class I restricted cytotoxic lymphocytes do not appear to play a significant role in resistance to infection in mice.

U S GOVERNMENT PRINTING OFFICE '391 0-664-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00325-12 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Respiratory Viruses in Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	James E. Crowe, M.D.	Clinical Assoc. (CO)	LID, NIAID
	Susan L. Hall, Ph.D.	Senior Staff Fellow	LID, NIAID
	Peter L. Collins, Ph.D.	Supvy. Microbiologist	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
BIOQUAL, Inc., Rockville (Bradbury); Georgetown University (London); Wyeth-Ayerst Research (Davis)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of a CRADA between LID/NIAID and Wyeth-Ayerst Research to develop a live attenuated RSV vaccine, three new candidate vaccines strains were developed and were found to be satisfactorily attenuated in chimpanzees and able to induce significant resistance to experimental RSV challenge. One candidate (*cp*RSV-248), derived by chemical mutagenesis of an incompletely attenuated cold-passaged RSV candidate vaccine strain, was *ts in vitro* and attenuated for the upper and lower respiratory tract of the chimpanzee as evidenced by a 10-fold and a 1,000-fold restriction of virus replication, respectively, in these sites. A further mutagenized derivative of this virus (*cpts* 248/404) was more *ts in vitro* and was more highly restricted in replication in the upper and lower respiratory tract of the chimpanzee, exhibiting a 10,000-fold reduction and 100,000-fold reduction, respectively, in these sites. The third candidate vaccine virus studied, *ts*1 NG1/20-A-4, was a more attenuated derivative of the incompletely attenuated *ts*1 NG1 mutant. The *ts*1 NG1/20-A-4 was similar to the *cpts* 248/404 *in vitro* and in chimpanzees. Infection with each of these three candidate live attenuated RSV vaccine mutants induced almost complete resistance to subsequent RSV challenge. These findings form the basis for our decision to proceed with the evaluation of these three candidate vaccine strains, as well as three other similar candidate strains, in humans. These clinical trials will begin this summer.

The attenuation phenotype of the bovine parainfluenza virus type 3 vaccine was found to be stable following replication of the virus in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00326-12 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Respiratory Viruses in Volunteers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	E. Kanta Subbarao, M.D.	Senior Staff Fellow	LID, NIAID
	Susan L. Hall, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*
PRI DynCorp., Rockville, MD (Potash); Johns Hopkins University, Baltimore, MD (Clements); St. Louis University, St. Louis, MO (Belshe); Vanderbilt University, Nashville, TN (Wright)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.6	1.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A live attenuated influenza A cold-adapted (*ca*) vaccine ($10^{6.0}$ $TCID_{50}$/vaccinee) was safe in infants less than six months of age. However, the immunogenicity of the vaccine was low in two-month-old infants with less than 35% responding to vaccine, whereas 90% of vaccinees 6- to 36-months-old developed antibodies. Maternally-derived antibodies appeared to suppress the immunogenicity of the vaccine in two-month-old infants. These findings suggest that it may be necessary to use a higher dose of vaccine (*e.g.*, $10^{7.0}$ $TCID_{50}$/vaccinee) in this age group to overcome the suppressive effect of maternal antibodies.

The bovine parainfluenza virus type 3 (PIV3) is currently being evaluated as a vaccine to prevent disease caused by human PIV3. At a dose of $10^{5.0}$ $TCID_{50}$, the BPIV3 was safe and immunogenic in seronegative children. The attenuation phenotype of BPIV3 was stable during prolonged replication in humans. A dose of $10^{5.0}$ $TCID_{50}$ represents more than 100 human infectious dose 50's of the virus. These observations indicate that this candidate vaccine is satisfactorily infectious, safe, and immunogenic in seronegative humans. Additional studies involving BPIV3 are currently underway in humans. Another candidate vaccine virus JS HPIV3 *cp*45 is also being evaluated in seronegative infants.

U.S. GOVERNMENT PRINTING OFFICE *991 0 864 909

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00327-12 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Studies of Parainfluenza Type 3 Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Susan L. Hall, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Shu-Yuan Xiao, M.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The JS strain of PIV3 is the wild type parent of three candidate live attenuated cold-passaged (*cp*) viruses; namely *cp*12, *cp*18, and *cp*45. In order to develop an understanding of the genetic basis of attenuation of the mutant viruses, analysis of the nucleotide and deduced amino acid sequence of the JS *wt* and *cp*12 and *cp*45 mutant viruses was undertaken. The sequence of the JS wild-type PIV3 and of the *cp*12 and *cp*45 mutants is completed. The most promising attenuated vaccine candidate, the *cp*45 virus, contains at least 18 nucleotide changes from the JS wild type virus. There were nine changes in *cp*12 and eight in *cp*45 that would most likely contribute to their phenotypes. For *cp*12, two were non-coding changes in regulatory regions, one in the 3' genome leader and one in the NP gene transcription start signal. The remaining seven changes resulted in amino acid substitutions, in NP, F, HN, and L. For *cp*45, two mutations were in a non-coding regulatory region, the 3' genome leader. The remaining six changes resulted in amino acid substitutions in F, HN, and L. Only two nucleotide substitutions were shared by *cp*12 and *cp*45, a C>T change in position 334 in P and a T>C change at position 1224 in HN. The change at position 334 is a non-coding change; however, interestingly, it is present in the Wash/47885/57 virus. The T>C change at position 1224 in HN specifies an amino acid change and may, therefore, be responsible for a shared phenotype between the *cp*12 and *cp*45 viruses.

We are using the cDNA clones that were employed to sequence the JS wild type and *cp*45 viruses to construct complete cDNA copies of each of these two viruses, a project that is 90% complete for the JS wild-type. A strategy is being developed to produce an infectious virus from RNA transcribed from the complete cDNA copy of PIV3 which will greatly accelerate our ability to produce live attenuated PIV3 vaccines.

U.S. GOVERNMENT PRINTING OFFICE 1991 0 864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00345-12 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Studies of Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	James Crowe, M.D.	Research Assoc. (CO)	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Peter Collins, Ph.D.	Microbiologist	LID, NIAID
	Mark Connors, M.D.	Research Assoc. (CO)	LID, NIAID
	Arun Kulkarni, Ph.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(if any)*
LIP/NIAID/NIH (Morse); Wyeth-Ayerst Research, Radnor, PA (Davis); The Scripps Research Institute (Burton); PRI, La Jolla, CA (Zebedee)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.7	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Significant progress has been made in the development of a live attenuated RSV vaccine that is being pursued under an LID/NIAID-Wyeth-Ayerst Research CRADA. Incompletely attenuated respiratory syncytial virus (RSV) mutants were subjected to mutagenesis in order to introduce additional mutations and a large series of more attenuated derivatives were generated. These further attenuated RSV mutants, which contain at least two to four attenuating mutations, were evaluated *in vitro* and *in vivo* in mice and chimpanzees. This extensive evaluation has led to the identification of six candidate RSV vaccine strains that collectively exhibit three distinct levels of attenuation *in vivo*. Virus suspensions of these six RSV vaccine candidates are being produced under GMP conditions for Phase I-II clinical studies in humans that should allow us to identify one or more mutants with the optimal level of attenuation, immunogenicity, protective efficacy and genetic stability.

Human monoclonal antibodies to RSV are being developed for passive immunoprophylaxis of infants and children who are at high risk for serious lower respiratory tract disease due to RSV as well as for immunotherapy. Two genetically distinct Fab fragments of RSV F glycoprotein-specific IgG, selected from a combinatorial library expressed on the surface of filamentous DNA bacteriophage, were shown to have high neutralizing activity for RSV in cell culture. One of these Fabs has been demonstrated to effect a 5,000-fold reduction in lung virus titer of RSV-infected mice following a single intranasal instillation of Fab. These observations suggest that human RSV-specific antibody Fab fragments grown in *E. coli* may be sufficient to treat serious disease in humans caused by RSV. In contrast, whole IgG antibodies (*i.e.*, Fc + Fab) constructed from the most active Fabs will be required for prevention of serious disease in high risk individuals.

The RSV M2 protein is a protective antigen in H-2^d mice and the resistance it provides to RSV challenge is mediated by a cytotoxic T-cell (CTL) response. Epitope mapping studies demonstrated that the RSV M2 protein contains an immunodominant K^d-restricted CTL epitope spanning amino acid residues 82-90 that is shared by subgroups A and B RSV strains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00368-11 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Analysis of the Genome of Respiratory Syncytial Virus (RSV)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Supvy. Microbiologist	LID, NIAID
Others:	Haim Grosfeld, Ph. D.	Visiting Associate	LID, NIAID
	Lili Kuo, Ph.D.	Visiting Fellow	LID, NIAID
	Juan Cristina, Ph.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are developing methods for introducing cDNA-encoded synthetic genomic RNA (vRNA) of human respiratory syncytial virus (RSV) into the viral replicative cycle. The ultimate goal is to produce infectious virus from a complete synthetic vRNA. This capability would provide an experimental system for analyzing the functions of RSV nucleic acid signals and proteins and would provide, for the first time, a method for producing defined viral mutants for pathogenesis studies and for live vaccines. cDNAs were constructed that encode vRNA analogs in which all of the viral genes have been deleted and replaced with the bacterial chloramphenicol acetyl transferase (CAT) marker gene and/or the luciferase marker gene. When transfected into tissue culture cells and complemented by infection with RSV, these truncated, negative-sense vRNA analogs were "rescued" in that they were expressed, amplified and packaged into infectious particles. Substantial progress has been made in identifying and characterizing *cis*-acting nucleotide signals important in rescue.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00372-11 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Roles of RSV Proteins in Host Immunity and Molecular Approaches to Vaccine Design

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Supvy. Microbiologist	LID, NIAID
Others:	Haim Grosfeld, Ph.D.	Visiting Scientist	LID, NIAID
	Lili Kuo, Ph.D.	Visiting Fellow	LID, NIAID
	Juan Cristina, Ph.D.	Visiting Fellow	LID, NIAID
	Mark Connors, M.D.	Clinical Associate (CO)	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID

COOPERATING UNITS *(if any)*

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SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are using recombinant DNA techniques in the development of vaccines to human respiratory syncytial virus (RSV). Previously, recombinant vaccinia viruses or adenoviruses were constructed that expressed individual RSV proteins including the major protective antigens, the F and G glycoproteins. Efficacy tests in rodents and monkeys provided highly encouraging results, but in chimpanzees (a fully permissive host for RSV) the recombinants proved to be insufficiently immunogenic and protective. Improvements in vector technology will be required before this approach will be feasible for RSV immunoprophylaxis. A second approach involves efforts to develop methods for producing infectious RSV from full length cDNA. This would make it possible to produce and characterize attenuated strains by direct genetic engineering. To date, we have successfully "rescued" a cDNA-encoded vRNA analog which is 49.3% of full-length and includes a foreign reporter gene under the control of RSV transcriptive signals. Standard infectious RSV was used as helper to provide proteins to complement the cDNA-encoded vRNA analog. This analog was packaged into infectious particles and was passaged five times in culture with undiminished efficiency. This provides support for the idea that a complete, nondefective cDNA-encoded vRNA can be rendered biologically active so as to yield infectious virus.

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U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00498-07 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis, Processing and Functions of the Proteins of Human RSV

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph. D.	Supvy. Microbiologist	LID, NIAID
Others:	Haim Grosfeld, Ph.D.	Visiting Associate	LID, NIAID
	Lili Kuo, Ph.D.	Visiting Fellow	LID, NIAID
	Juan Cristina, Ph.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We would like to have a detailed picture of the processing, functions and assembly of the proteins of human respiratory syncytial virus (RSV). In previous years, we determined the primary structures of the viral proteins from sequencing cloned viral genes. Comparison of different RSV strains identified various putative functional domains and highlighted certain protein regions that were either tolerant or intolerant of amino acid diversity. The folding, processing and oligomerization of the F, G and SH transmembrane glycoproteins were studied in detail using biochemical and cDNA mutation/expression techniques. We are now trying to develop a new system for analyzing the viral proteins based on complementation in tissue culture of analogs of viral genomic RNA that express a reporter gene. This would allow us to identify the viral proteins required for the major steps in the viral replicative cycle, in particular transcription, replication and virus production. The system could be used to perform detailed structure-function studies of individual proteins involved in all stages of the replicative cycle. We also use vectors expressing the appropriate proteins to activate and rescue synthetic complete vRNA encoding infectious virus.

U S GOVERNMENT PRINTING OFFICE 1991 0 864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00370-11 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis Studies of Simian Immunodeficiency Virus (SIV) Infection of Macaques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Vanessa Hirsch, D.V.M., D.Sci.	Visiting Scientist	LID, NIAID
Others:	Simoy Goldstein, Ph.D.	Visiting Scientist	LID, NIAID
	Noreen A. Hynes, M.D.	Senior Investigator	LID, NIAID
	Barbara Campbell, Ph.D.	Staff Fellow	LID, NIAID
	William R. Elkins, D.V.M.	Senior Veterinarian	LID, NIAID
	Harold Ginsberg, M.D.	Fogarty Scholar	LID, NIAID
	Raymond Langley, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*
LIR, NIAID, NIH, Bethesda, MD (Fauci, Fox); The Ohio State University (Johnson); The Henry Jackson Foundation (Zack, Lewis); Southwest Foundation for Biomedical Research (Allan); Yerkes Regional Primate Research Center (McClure); Los Alamos National Laboratory (Myers)

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Laboratory of Infectious Diseases

SECTION
Immunodeficiency Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.5	6.5	3.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major emphasis of this project was the study of SIV pathogenesis in experimentally-infected macaques, primarily utilizing strains derived from sooty mangabeys (SIVsm). However, pathogenicity of SIV from Sykes' monkeys (SIVsyk) and SIVagm from African green monkeys was also evaluated. The principle goal was to map viral determinants of pathogenesis, and to study correlates of disease progression.

Initial studies characterizing genetic determinants of pathogenicity focused on the acutely lethal SIVsm variant, SIVsmm/PBj. The pathogenic determinants of this virus are complex, and multigenic, although the envelope was identified as a critical viral determinant. We expanded these studies to SIVsm clones derived from an immunodeficient macaque to study viral factors contributing to the more traditional AIDS syndrome. This study of SIVsm clones derived from the spleen of an immunodeficient SIV-infected pig-tailed macaque identified a region analogous to the V3 loop of HIV-1 envelope to be responsible for determining tropism. Naturally-occurring variation in this region determined whether viruses infected macaque lymphocytes and/or macrophages and this tropism appeared to be linked to pathogenicity.

Early virologic events in SIV pathogenesis were studied in four macaques inoculated with SIVsm/E543 PBMC. Molecular analysis of viral DNA in PBMC demonstrated that each macaque was infected with a distinct variant. Variation in the envelope in PBMC, plasma, CSF, and lymph nodes samples are being evaluated.

A SIVagm strain (SIVagm9063) was isolated and molecularly cloned after experimental passage through a pig-tailed macaque. This isolate induced immunodeficiency in experimentally infected pig-tailed macaques. Two infectious, molecular clones have been obtained and are being tested for pathogenicity in macaques.

U S GOVERNMENT PRINTING OFFICE 1991 0 864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00616-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Feline Immunodeficiency Virus Infection as a Model for Human AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Raymond Langley, Ph.D.	Visiting Associate	LID, NIAID
Others:	Vanessa Hirsch, D.V.M., D.Sci.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
NCI (O'Brien); Florida Fresh Water Fish and Game Commission (Roelke); UpJohn (Olmsted)

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Laboratory of Infectious Diseases

SECTION
Immunodeficiency Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1992-93

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00686-01 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Vaccine Studies of Simian Immunodeficiency Virus (SIV) Infection of Macaques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Vanessa Hirsch, D.V.M., D.Sci.	Visiting Scientist	LID, NIAID
Others:	Simoy Goldstein, Ph.D.	Visiting Scientist	LID, NIAID
	Noreen A. Hynes, M.D.	Senior Investigator	LID, NIAID
	Barbara Campbell, Ph.D.	Staff Fellow	LID, NIAID
	Malcolm Lawson, Ph.D.	Visting Fellow	LID, NIAID
	William R. Elkins, D.V.M.	Senior Veterinarian	LID, NIAID

COOPERATING UNITS *(if any)*
LVD, NIAID, NIH, Bethesda, MD (Moss, Sutter); GeneLabs (Fuerst); Ohio State Univ. (Johnson); The Henry Jackson Found. (Zack, Lewis); Yerkes Reg. Primate Res. Ctr. (McClure); Div. Molec. Virol. & Immunol. (Gerin); Vanderbilt Univ. (Montifiori); Bristol Myers Squibb (Haigwood); Life Technol. (Jessee); Chiron Corp. (Van Nest)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Immunodeficiency Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major emphasis of this work was the use of the SIV/macaque model of AIDS for the evaluation of potential vaccine strategies. A number of different strategies for inducing an SIV-specific immune response have been investigated. An initial strategy utilizing whole inactivated SIV (WI-SIV) derived from a SIV proviral clone was highly successful in preventing infection after an homologous or heterologous cell-free SIV challenge. However, this protection appears to be mediated by antibodies generated to human cell proteins in the viral immunogen that was propagated in a human cell line. Thus protection was not observed following challenge with SIV grown in macaque PBMC. The basis for protection of monkeys with WI-SIV was analyzed by immunizing eight animals with WI-SIV and subsequently challenging these animals with either virus grown in human cells (SIV-hu) or macaque PBMC (SIV-mac). All the animals challenged with SIV-hu were resistant to infection, whereas all animals challenged with SIV-mac became infected. A similarly immunized group of five macaques were also challenged with PBMC-associated SIV and as predicted from the previous experiment all became infected. However, long-term evaluation showed that the immunized animals survived longer than the naive controls suggesting that immunization may modify the course of disease.

A neutralization assay for SIV was established by developing an adherent macaque CD4+ cell line. This cell line is susceptible to a wide range of SIV isolates, and virus infectivity and neutralizing antibody titers can be determined by the enumeration of infected cells that are detected by immunoperoxidase labeling with SIV-specific antiserum.

We constructed: (i) vaccinia virus recombinants that express the SIV gagpol and env, and (ii) integrase deletion mutants of SIV. These recombinants and SIV mutants were analyzed for expression of SIV proteins. In transient transfection assays a vaccinia virus recombinant expressing envelope from a synthetic promoter was shown to produce 10- to 20-fold more protein than constructs under the control of a 7.5K promoter. Vaccine trials utilizing these vaccinia virus recombinants, and genetic immunization are in progress.

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00308-07 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
In Vitro and *In Vivo* Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS (*If any*)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

INACTIVE 1992-93

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00311-12 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Search For New Hepatitis Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert H. Purcell. M.D.	Head, HV Section	LID, NIAID
Others:	Patrizia Farci, M.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

Hospital for Sick Children, Toronto, Canada (Phillips); Howard University Medical School (Sewchand)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.64	0.64	1.00

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1989 a possible paramyxovirus etiology for giant cell hepatitis was proposed, based on electron micrographic changes seen in the livers of patients with this disease. Collaborative studies, including attempts to transmit the disease to primates, are in progress.

Patients with thalassemia in Sardinia, Italy, receive monthly blood transfusions as therapy. Many of these patients have developed chronic hepatitis. Most cases could be identified as hepatitis B or hepatitis C, but some lacked markers of either of these viruses. These patients are being studied for evidence of previously unrecognized hepatitis viruses.

Patients with fulminant non-A, non-B hepatitis generally do not have evidence of infection with any recognized hepatitis virus. Only about 10% can be identified as having fulminant hepatitis C. The remaining 90% remain a diagnostic enigma and may be infected with one or more previously unrecognized viruses. In collaborative studies, we are attempting to transmit the disease to primates.

The objectives of this project are to identify and characterize new etiologic agents of hepatitis and to develop useful assays for diagnosis of infection and seroepidemiologic studies. A longer term objective is the development of passive and active immunoprophylaxis for these important human pathogens.

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00314-12 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Woodchuck Virus: Molecular Biological Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Roger Miller. Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Hong-Shu Chen, M.D., Ph.D.	Visiting Associate	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
	Atshushi Shimoda, M.D.,D.M.S.	Visiting Scientist	LID, NIAID
	Fumihiko Sugata. M.D., D.M.S.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

Division of Molecular Virology & Immunology, Georgetown University, Washington, DC (Gerin); New York State College of Veterinary Medicine (Tennant)

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Laboratory of Infectious Diseases

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Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.17	0.33

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. Thus, WHV infection of woodchucks provides a relevant and convenient model for understanding HBV infections of humans. Four major areas of study were pursued this year. First, we extended the mutagenesis of the WHV X gene and found that nine of ten such mutants were replication negative in a woodchuck liver transfection assay. Second, we extended our characterization of the X gene promoter and have mapped the location of the promoter and defined the essential nucleotides in this *cis*-acting element. Third, we initiated experiments on characterization of the WHV pregenome promoter and have identified two separate promoters. One promoter is located on the minus strand of DNA while the other is on the plus strand of DNA. Thus, we obtained evidence for a bi-directional promoter in WHV. Fourth, the complete nucleotide sequence of the New York strain of WHV was determined. Also, the nucleotide sequence of selected gene regions of over 100 independent clones was determined to ascertain the extent of sequence variation among WHV genomes from a chronically infected woodchuck. We sequenced over 70,000 nucleotides and found a mutation rate of 1.6 changes per 10,000 nucleotides analyzed. Overall, significant progress has been made in the characterization of WHV gene products and *cis*-acting elements which must be understood in order to understand ultimately the mechanism of oncogenesis of this virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00530-06 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular and Computer Analysis of the Hepatitis B Virus Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Norio Ogata, M.D., D.M.S.	Visiting Scientist	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.47	0.33

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis B virus (HBV) is the prototype of the hepadnavirus family, which includes hepatotropic viruses that infect at least six separate species. Infection with HBV causes polymorphic liver diseases including acute or fulminant hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Over 300 million people throughout the world are chronically infected with HBV and are at high risk of developing hepatocellular carcinoma, one of the world's most prevalent forms of cancer.

The major surface (S) protein of the envelope of HBV contains an antigenic determinant in a hydrophilic domain, designated the *a* determinant, that is believed to contain an essential epitope that induces and reacts with neutralizing or protective antibodies. Two major areas of study were pursued this year to characterize the importance of this epitope in virus infection. First, the protective efficacy of a human monoclonal antibody directed against the *a* determinant was investigated in a chimpanzee. A single high dose of antibody was administered intravenously followed by challenge with wild-type virus. The passively acquired antibody could be detected for 40 weeks during which there was no evidence of viral replication. The chimpanzee subsequently developed acute hepatitis B approximately 1 year after challenge. Thus, immunization with a monoclonal antibody directed against the major putative neutralization epitope of the HBV S protein significantly delayed but did not protect against infection.

Second, we continued our studies of a putative neutralization escape mutant of HBV that emerged in individuals who were vaccinated against hepatitis B. This virus exhibited antigenic variation within the *a* determinant. In each case an identical nucleotide substitution occurred that resulted in a single amino acid change in this determinant. Since the emergence of this mutant HBV raised questions about the efficacy of the existing recombinant vaccines for hepatitis B, two licensed vaccines were investigated for their ability to protect chimpanzees against challenge with the S gene mutant. Both recombinant vaccines provided protection against challenge with the mutant suggesting that properly vaccinated individuals are not at risk of infection by the S gene mutant virus.

U.S. GOVERNMENT PRINTING OFFICE *1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00569-04 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determination of Genetic Markers of Virulence & Adaptation to Cell-Culture of Hepatitis A Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Ann Funkhouser, M.D.	Research Associate (CO)	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
	Gopa Raychaudhuri, Ph.D.	IRTA Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.39	3.14	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis A virus (HAV) is a picornavirus with a single-stranded RNA genome of approximately 7500 nucleotides. The wild-type strain of HAV grows poorly in cell-culture, generally is not cytopathic, and yields a low titer of virus. A cell-culture adapted mutant has been isolated which grows significantly more efficiently in cell-culture and which is attenuated for marmosets and chimpanzees. The objectives of this project are to determine the genetic basis for virulence and adaptation to cell culture of HAV in order to develop a strain of HAV suitable for an attenuated vaccine.

1. Four mutations in the 5' noncoding region were evaluated for their effect on viral growth in CV-1 cells.
2. The entire genome of a vaccine strain of HAV was sequenced.
3. New viable chimeric viruses were constructed which contained genomic regions from two or more HAV strains including a human virulent strain, a human attenuated strain, a vaccine strain and a simian strain.
4. Infection of marmosets with chimeric viruses suggested that the 2C gene may be the most significant, but not the sole, determinant of virulence.
5. A modified radioimmunofocus assay was developed for HAV plaque analysis.

U.S. GOVERNMENT PRINTING OFFICE 1991 0 864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00570-04 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Hepatitis C Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
	Patrizia Farci, M.D.	Visiting Scientist	LID, NIAID
	Jens Bukh, M.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

National Institute of Health, Tokyo, Japan (Shimizu)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.97	0.83

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis C virus (HCV) is an important human pathogen that is strongly associated with transfusion related non-A, non-B hepatitis and hepatocellular carcinoma (HCC). HCV accounts for up to 25% of community-acquired hepatitis and over 90% of transfusion-associated hepatitis in the United States. The recent cloning and sequencing of the genome of HCV led to the development of specific serologic tests for antibody to HCV that utilized recombinant-derived viral antigens. These tests have been useful for diagnostic and screening purposes but they shed little light on immunity to HCV, in part because currently available antigens are expressed from parts of the HCV genome that encode nonstructural or internal structural proteins. Furthermore, the finding of genetic heterogeneity of the HCV genome, especially in the genes encoding the envelope proteins, suggests that there may be heterogeneity of viral envelope proteins similar to that seen in human immunodeficiency viruses. Such a finding would bode ill for attempts at vaccine development. Numerous studies have documented an association between the presence of antibodies to HCV and development of HCC in the absence of evidence of prior infection with hepatitis B virus (HBV). In this project we have used a sensitive and specific cDNA polymerase chain reaction assay to detect HCV RNA in serum samples from 128 unselected southern African blacks with HCC. We found that a significant number of such patients had evidence of current HCV infection, but not of current HBV infection, suggesting that infection with HCV plays a role in the development of HCC in this population. Other major findings were: (i) identification of seven new genetic types of HCV; (ii) successful replication of HCV in tissue culture; and (iii) biophysical characterization of light and heavy forms of HCV.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00596-03 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vivo and *In Vitro* Studies of Hepatitis E Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Sergei Tsarev, Ph.D., D.Sci.	Visiting Fellow	LID, NIAID
	Shu-Rong Yin, M.D.	Visiting Fellow	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.39	2.14	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to increase our understanding of hepatitis E virus (HEV) and related viruses with the goal of designing strategies for their control.

1. The suitability of a number of different species of nonhuman primates as animal models for the study of hepatitis E has been evaluated.
2. We have studied in detail the natural history of HEV infection in nonhuman primates.
3. The infectivity titer of the Sar-55 strain of HEV was determined in cynomolgus monkeys.
4. Strains of HEV from different geographic regions have been compared by sequence analyses.
5. We have sequenced the entire genome of a Chinese strain of HEV.
6. Seroepidemiologic studies of HEV have been carried out in Pakistan, Saudi Arabia and Belize with new serologic tests (ELISA and Western blot).
7. Plasma from experimentally infected monkeys has been collected and is being evaluated for passive immunoprophylaxis. Novel methods of expressing the structural protein(s) of HEV have been used to generate antigens suitable for vaccine development.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00500-06 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Processing and Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NS1

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Barry Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID
	Lewis Markoff, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1992-93

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00531-05 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Analysis of Dengue Nonstructural Proteins NS2B and NS3

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Barry Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1992-93

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00571-03 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Amino Acid Substitution at the NS1-NS2A Cleavage Junction of Dengue Virus Polyprotein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michèle Pethel	Microbiologist	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1992-93

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00572-03 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Processing of Dengue Virus Polyprotein NS3-NS4A-NS4B-NS5

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Barry Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID
	Annie Cahour, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1992-93

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00597-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Construction and Characterization of Chimeric Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1992-93

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864 039

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00598-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dengue Type 4 Virus Mutants Restricted in Polyprotein Processing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hiroshi Kawano, M.D.	Visiting Associate	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1992-93

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00600-03 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Engineering Viable Dengue Virus 3' Noncoding Region Deletion Mutants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ruhe Men, M.D.	Visiting Associate	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dengue type 4 virus (DEN4) genome contains 384 nucleotides in the 3' noncoding region (NCR). Sequence analysis indicates that there are two types of conserved sequences, designated CS-1 and CS-2, that are also found in other mosquito-borne flaviviruses. The last 82 nucleotides in DEN4 3' NCR form a stable stem-and-loop secondary structure similar to that found in other flaviviruses. We have engineered a series of cDNA constructs containing deletions ranging from 30 to 202 nucleotides in length in the 3' NCR. Mutant RNA transcripts made from these DNA constructs were tested for infectivity by transfecting permissive tissue culture cells. Viable dengue virus was recovered from mutant DNA constructs containing a deletion in one or the other or both of the two CS-2 sequences. In contrast, the last 112 nucleotides, including the stem-and-loop structure at the 3' end, the preceding CS-1 sequence, and 1~7 nucleotides upstream of CS-1 are essential for infectivity of dengue virus. Many viable deletion mutants constructed in this manner were stable and produced plaques of reduced size on infected C6/36 cells compared to wild type virus. Analysis of viral growth in primate-derived LLC-MK_2 cells revealed that most deletion mutants produced plaques that were slow to develop. Furthermore most mutants grew slowly and attained a lower titer than parental wild type virus. These observations indicate that these DEN4 deletion mutants were restricted in growth. An exception was found for deletion mutant (Δ3' 303-183) which produced small plaques on C6/36 cells but grew to a high titer similar to that of parental wild type virus. This deletion mutant plus four other growth-restricted deletion mutants were selected for evaluation of infectivity and immunogenicity in rhesus monkeys and compared to the wild type virus. For reasons presently not understood, neither deletion mutants nor wild type virus induced viremia in monkeys. Nevertheless, mutant (Δ3' 303-183) induced an antibody response, measured by radioimmunoprecipitation and virus neutralization *in vitro*, similar to that of wild type virus. In contrast, other DEN4 3' deletion mutants elicited low to moderate levels of antibody. The immunogenicity of these 3' deletion mutants in monkeys appeared to be directly related to the level of growth attained in infected LLC-MK_2 tissue culture cells.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00637-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of New Approaches to Vaccines Against Tick-Borne Encephalitis Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Alexander Pletnev, Ph.D., D.Sci.	Visiting Scientist	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
Novosibirsk Institute of Bioorganic Chemistry; USAMRIID (Huggins)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In a recent study, full-length cDNAs of chimeric tick-borne encephalitis virus/dengue type 4 virus (TBEV/DEN4) constructs were prepared and RNA transcripts of these cDNAs were shown to be infectious for permissive simian cells in culture. Two new viable chimeric flaviviruses were recovered that contained TBEV structural protein CME or ME genes with the remaining genes derived from DEN4. Studies in which mice were inoculated intracerebrally with the ME chimeric virus indicated that it retained the neurovirulence of its TBEV parent from which its M and E genes were derived. However, unlike parental TBEV, the chimeric virus did not produce encephalitis when mice were inoculated peripherally, indicating a loss of neuroinvasiveness. In the present study, the ME chimeric virus (vME) was subjected to mutational analysis in an attempt to reduce or ablate neurovirulence manifest when virus is inoculated directly into the brain. Three distinct mutations were identified that were independently associated with a significant reduction of mouse neurovirulence of the ME chimera. These mutations ablated: (i) the TBEV PreM cleavage site which is required for proper processing of M protein; (ii) the TBEV E (envelope glycoprotein) glycosylation site; or (iii) the first DEN4 NS1 (non-structural protein one) glycosylation site. In contrast, ablation of the second DEN4 NS1 glycosylation site or the TBE PreM glycosylation site or amino acid substitution at two positions in the TBEV E protein effected a moderate increase in neurovirulence. The only conserved feature of the 3 attenuated mutants was restriction of virus growth yield in both simian and mosquito cells. Following parenteral inoculation, these attenuated mutants induced complete resistance in mice to fatal encephalitis caused by subsequent challenge with the highly neurovirulent ME chimera. Our success in ablating neuroinvasiveness and neurovirulence manifest when TBEV is inoculated directly into the brain, suggests that the strategy used to achieve these effects might yield a live attenuated TBEV vaccine.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00638-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Dengue and Other Flavivirus Proteins Using Baculovirus as a Vector

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Ruhe Men, M.D.	Visiting Associate	LID, NIAID
	Chun-Fa Zhang, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*
WRAIR, Washington, DC (Summers, Eckels, Dubois, Sumiyoshi)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Earlier studies of a vaccinia virus recombinant expressing dengue type 4 virus (DEN4) envelope protein (E) indicated that a C-terminally truncated E, approximately 80% in length, was more immunogenic and protective in mice than the full-length E. The increase of immunogenicity and protective efficacy associated with the specific truncation of E has implications for the development of effective subunit vaccines for dengue viruses as well as other flavivirus diseases. In an effort to explore these implications, we constructed recombinant baculovirus expressing 80%E of dengue virus or other flaviviruses using the high yielding baculovirus-insect cell system. The baculovirus-expressed 80%E proteins of DEN4 or DEN2 were secreted into the fluid medium of infected insect sf9 cells and were stable. The relative immunogenicity of the intracellular products of b(DEN4, 80%E) and b(DEN4, 93%E) and secreted 80%E in concentrated medium was determined by inoculating mice with the same amounts of these materials and assessing their antibody response. All mice inoculated with secreted 80%E developed antibodies to the E protein, whereas mice inoculated with a lysate of cells infected with b(DEN4, 93%E), b(DEN4, 80%E), or wild-type baculovirus little if any E-specific antibody as determined by radioimmunoprecipitation. Based on these observations we evaluated baculovirus-expressed DEN4 80%E in monkeys to determine if immunogenicity of DEN E had been enhanced. Groups of rhesus monkeys were inoculated 3 times intramuscularly with: (i) a lysate of cells infected with b(DEN4, 80%E), (ii) concentrated fluid medium from the cultures of these cells, (iii) a lysate of cells infected with b(DEN4, 93%E), (iv) a lysate of cells infected with wild type baculovirus, or (v) concentrated medium from a culture of wild type baculovirus infected-cells. The antibody response to immunization as determined by plaque reduction neutralization was low in each group; two monkeys that received secreted 80%E had a titer of 1:20 while others developed a titer of 1:10, or were negative. Tests for post challenge viremia, which included amplification of viral isolates on mosquito C6/36 cells followed by plaque assay on the same cells, could not be interpreted because unimmunized control monkeys did not regularly become viremic. Although the protective efficacy of the recombinant baculovirus products could not be evaluated in monkeys, it was evident that these materials are at best weakly immunogenic in monkeys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00681-01 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Determination of Genetic Loci of Dengue Type 2 Virus Neurovirulence in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dengue virus, a flavivirus, causes human disease throughout tropical regions of the world. There are four serotypes; infection with one produces immunity only to that type. Serotype specificity is determined by the viral structural proteins, capsid (C), pre-membrane (pre-M) and envelope (E). The dengue viral genome, an 11 kilobase strand of positive-sense RNA, contains the three structural protein genes followed by a series of 7 non-structural protein genes. Earlier, we constructed a full-length cDNA copy of the entire dengue 4 (D4) genome, RNA transcripts from which were infectious when transfected into mammalian cells. We subsequently replaced the C-preM-E or preM-E genes of the full-length clone with the structural protein genes of dengue type 1 (D1) or type 2 (D2) virus, and used these templates to create chimeric D1/4 and D2/4 viruses. Two D2/D4 chimeric viruses have been constructed, one using the preM-E genes of a D2 New Guinea C strain virus isolated from a human patient (D2 NGC prototype) the other using the C-preM-E or preM-E genes of a mutant of that virus selected for mouse neurovirulence by serial passage in mouse brain (D2 NGC neurovirulent). D4 virus derived from the full-length clone and the chimeric (D2 ME prototype)/D4 virus were not neurovirulent in mice. Chimeric viruses (D2 NGC CME neurovirulent)/D4 and (D2 NGC ME neurovirulent)/D4 were both neurovirulent for suckling mice, but did not produce disease in 6-week-old mice, whereas D2 NGC neurovirulent was lethal at low dose. This indicated that some but not all of the genetic loci responsible for neurovirulence are located in the structural protein genes. Sequence analysis of the cloned C-preM-E genes of prototype and neurovirulent D2 NGC revealed 7 nucleotide differences in the neurovirulent mutant which should result in amino acid changes. A series of chimeric viruses have been constructed in which these mutations were introduced on a background of the prototype sequence. Testing of these chimeras in mice has been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00682-01 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Intertypic Chimeric Dengue Viruses as Candidate Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dengue virus, a flavivirus, causes human disease throughout tropical regions of the world. There are four serotypes; infection with one produces immunity only to that type. An effective dengue vaccine must protect against all four dengue serotypes. Serotype is a property of the viral structural proteins, particularly the viral envelope glycoprotein (E). The viral genome, an 11 kb strand of positive-sense RNA, contains the three structural protein genes, capsid (C), pre-membrane(pre-M) and envelope (E), followed by a series of 7 non-structural protein genes. Earlier, we constructed a full-length CDNA copy of the entire dengue 4 (D4) genome, which yields infectious full-length RNA transcripts. We subsequently replaced the C-preM-E genes of the full-length clone with the C-preM-E genes of D1 virus or the C-preM-E or preM-E genes of D2 virus, and used these templates to create chimeric D1/4 and D2/4 viruses. Cells infected with these chimeras produce authentic D1 or D2 structural proteins. We have tested two chimeric viruses in rhesus monkeys as a first step in vaccine evaluation. In the first experiment, monkeys were immunized with either D4 virus, wild-type D1 or D2 virus, or chimeric D1(C-preM-E)/D4 or D2(preM-E)/D4. Monkeys inoculated with either chimera developed less viremia compared to animals inoculated with wild-type D1 or D2 virus. Furthermore, their D1- or D2-neutralizing antibody response was intermediate between that of D4 virus recipients and wild-type D1 or D2 virus recipients. The D4 control group was not protected against wild-type D1 or D2 virus challenge, while monkeys immunized with D2/D4 or wild-type D2 virus were fully protected against D2 virus challenge. Two of four monkeys inoculated with D1/D4 were fully protected against D1 virus challenge, while one other animal was partially protected. The remaining animal in the group was observed to be fully susceptible. In a second experiment, monkeys were immunized with D4 virus or a mixture of the chimeric D1/D4 and D2/D4 viruses, as a prototype bivalent vaccine. Sera from this experiment are currently being evaluated. A set of suitably engineered chimeric viruses might serve together as a safe and effective dengue vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00683-01 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Determinants of Dengue Virus Mouse Neurovirulence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Hiroshi Kawano	Visiting Associate	LID, NIAID
	Kazufumi Hiramatsu, M.D.	Visiting Associate	LID, NIAID
	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

University of Hawaii, Honolulu, HA (Rosen)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mouse-adapted dengue type 4 virus (DEN4) strain H241 is highly neurovirulent for mice, whereas its non-mouse adapted parent is rarely neurovirulent. The genetic basis for neurovirulence of the mouse-adapted mutant was studied by comparing intratypic chimeric viruses that contained the three structural protein genes of the parental virus or the neurovirulent mutant on the background sequence of non-neurovirulent DEN4 strain 814669. The chimera that contained the three structural protein genes of mouse neurovirulent DEN4 H241 proved to be highly neurovirulent in mice, whereas the chimera that contained the corresponding genes of its non-mouse adapted parent was not neurovirulent. These observations indicate that most of the genetic loci for neurovirulence of the DEN4 mutant map within the structural protein genes. A comparison of the amino acid sequences of the parent and its mouse neurovirulent mutant revealed that there were only five amino acid differences in the structural protein region and three of these were located in the envelope (E) glycoprotein. Analysis of the chimeras which contained one or two of the variant amino acids of the mutant E sequence substituting for the corresponding sequence of the parental virus identified two of these amino acid changes as important determinants of mouse neurovirulence. First, the single substitution of Ile for Thr_{155} which ablated one of the two conserved glycosylation sites in DEN E yielded a virus that was almost as neurovirulent as the mouse-adapted mutant. Thus, the loss of an E glycosylation site appears to play a role in DEN4 neurovirulence. Second, the Leu for Phe_{401} substitution also yielded a neurovirulent virus but it was less neurovirulent than the glycosylation mutant. Also, a comparison of the amino acid sequences in the structural protein region of DEN4 strain H241 and strain 814669 isolated 25 years apart indicates that genetic variation produced mostly non-conservative changes which could explain the observed antigenic difference between the two virus strains.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00333-12 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Longitudinal and Cross-Sectional Studies of Viral Gastroenteritis in Infants & Young Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
	Judy F. Lew, M.D.	Sr. Research Investigator	LID, NIAID
	Mariam W. Watson, B.S.	Biologist	LID, NIAID

COOPERATING UNITS *(if any)*
Children's Hospital National Medical Center, Washington, DC (Kim); Univ. of Massachusetts Medical School, Worcester, MA (Herrman and Blacklow); John Radcliffe Hospital, Oxford, United Kingdom (Kurtz and Lee)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.41	0.08	0.33

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because of the availability of stool and serum specimens from several large-scale studies carried out previously in infants and young children, we are in the enviable position of being able to elucidate the natural history of infection when new viral agents are discovered or as new parameters of infection with known viral agents are recognized. Two major studies carried out by the LID in collaboration with others provide the focus of this project. One is a longitudinal study (1955-1969) at Junior Village, a welfare institution for homeless but otherwise normal children, and the other a cross-sectional study (1974-1991) of children hospitalized with gastroenteritis at Children's Hospital National Medical Center, Washington, DC. Our goal in the Junior Village studies is to investigate the natural history of rotavirus and Norwalk infection in a longitudinal setting, whereas in the Children's Hospital study it is to determine the importance of Norwalk virus and astrovirus as agents of severe gastroenteritis requiring admission to the hospital. There is evidence from several studies including this one that indicates that the Norwalk virus and the astrovirus each cause infections in infants but, as yet, their importance as etiologic agents of severe gastroenteritis is not certain. Such information must be elucidated in order to set priorities for vaccine development.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00339-12 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and Serotypic Characterization of Human and Animal Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*

Centers for Disease Control (Gentsch), Akita University, Japan (Nakagomi)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.83	0.25	1.58

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human rotavirus strain 116E which was originally isolated from an asymptomatic infant in India was shown to share VP7 specificity with human rotavirus serotype 9 (prototype strain W161) and VP4 specificity with bovine rotavirus strain B223 (that belonged to VP7 serotype 10). Strain Ro1845 (VP7 serotype 3) which was isolated from a diarrheic child in Israel was found to share VP4 specificity with canine rotaviruses. In order to analyze interactions between the two rotavirus outer capsid proteins VP4 and VP7, various human x human rotavirus reassortants were generated, each of which had the VP7 specificity of the serotype 2 DS-1 strain. Guinea pig hyperimmune antiserum to each of the reassortants was also generated. Analysis of the VP4-VP7 interaction by cross-neutralization is in progress.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00340-12 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Genetic Studies of Rotavirus Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Ronald Jones	Biologist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.17	0.17	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

By using a semi-homologous system of gnotobiotic newborn pigs and virulent porcine rotavirus (strain SB-1A) and avirulent human rotavirus (strain DS-1) and their reassortants, we demonstrated last year that: (i) the 3rd (VP3), 4th (VP4), 9th (VP7), or 10th (NS28) porcine rotavirus gene each plays an important independent role in virulence of rotavirus infection in piglets, and (ii) all four of the porcine rotavirus virulence-associated genes are required for the induction of diarrhea and virus shedding in piglets. In addition, it was found that the titer of serum neutralizing antibodies of piglets that were given a single-3rd-gene-substitution reassortant was four-fold lower than that of piglets administered a single-10th-gene-substitution reassortant. With this information as background, efforts have been made to generate various human x bovine rotavirus reassortants, each of which has both VP4-encoding and VP7-encoding genes from human rotavirus and at least the 10th gene from bovine rotavirus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00341-12 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Live Attenuated Rotavirus Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

Institute of Biomedicine, Caracas, Venezuela (Pérez-Schael); Univ. of Rochester (Madore, Christy); Johns Hopkins Univ. (Midthun); Univ. of Tampere (Vesikari, Ruuska); Institute of Nutrition, Peru (Lanata); Vanderbilt Univ. (Wright); Wyeth-Ayerst Res. (Davidson, Zito); Secretech, Inc. (Schafer); Dyn-Corp-PRI (Potash)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.33	0.17

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses have consistently been shown to be the single most important etiologic agents of severe diarrhea in infants and young children in both developed and developing countries, being responsible for 30-50% of such illnesses. In the United States, rotaviruses are estimated to cause about 110,000 hospitalizations annually for a total of 583,000 hospital days and about 75-150 deaths. In the developing countries, however, rotaviruses are estimated to cause over 17 million cases of moderately severe or severe diarrhea and over 870,000 deaths annually. There is an obvious need for a rotavirus vaccine in both developed and developing countries that is aimed at preventing severe rotavirus diarrhea during the first two years of life when the disease takes its greatest toll. The "Jennerian" approach to vaccination, which involves the use of a live vaccine virus strain derived from a non-human host, has been evaluated in clinical trials using surrogate rotavirus strains of bovine origin (by others) or of rhesus monkey origin by us. This approach has had limited success because serotype-specific immunity against each of the four clinically important human rotavirus VP7 serotypes could not be achieved consistently in infants less than six months of age with such a monovalent vaccine. We are currently pursuing a modified "Jennerian" approach in which a quadrivalent rotavirus vaccine containing: (i) rhesus rotavirus (RRV) (VP7 serotype 3), and (ii) three human rotavirus-RRV reassortants, each possessing ten RRV genes and a single human rotavirus gene that encodes VP7 serotype 1, 2, or 4 specificity, is given orally at two months of age. Placebo-controlled field trials with individual reassortant vaccines or the quadrivalent vaccine have been completed or are in progress in over 9,500 infants and young children in the United States or overseas. The modified strategy appears to be quite promising with regard to achieving the goal of preventing the serious consequences of rotavirus infection, namely severe diarrhea. A significant reduction in the incidence of rotavirus diarrhea in vaccinees when compared to placebo recipients has been observed in most, but not all, of the studies.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00342-12 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)

Epidemiology Division, US Naval Medical Research Institute, Rockville; Vanderbilt University (Wright)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.59	0.42	0.17

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Historically, the electron microscope has played an indispensable role in the discovery of viral gastroenteritis agents. Two major groups of gastroenteritis viruses--the 27nm Norwalk virus and the 70nm human rotavirus--were discovered at the Laboratory of Infectious Diseases (LID) and in Australia, respectively, (as well as the hepatitis A virus in the LID by investigators using electron microscopic techniques). These agents were discovered without the use of an *in vitro* tissue culture system, because they could not be grown from clinical specimens in cell culture. Although the electron microscope is of historic interest, it still plays an indispensable role in the study of these gastroenteritis viruses despite the availability of second and third generation tests for the detection of the Norwalk group of viruses and the rotaviruses. It is also the most rapid diagnostic method for detection of rotavirus from a clinical specimen and is the only method available for detecting infection with certain fastidious 27nm or similar small round virus-like particles from individuals with epidemic nonbacterial gastroenteritis. In addition, it is the only method that is capable of detecting all known gastroenteritis viruses (*e.g.*, group A or non-group A rotavirus, Norwalk-like viruses, adenoviruses, astroviruses, caliciviruses) by examination of a single stool specimen. It is also important in studies of baculovirus-expressed antigens of Norwalk virus and of hepatitis E virus, and it enables the characterization of the antigenic relationships of fastidious viruses by immune electron microscopy (IEM).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00343-12 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Norwalk Virus and Related Norwalk-Like Viruses in Viral Gastroenteritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Judy Lew, M.D.	Sr. Research Invest.	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*

Baylor College of Medicine (Estes); U.S. Naval Medical Research Institute (Hyams); University of Tampere, Finland (Vesikari)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.42	0.42	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The association of Norwalk virus and related 27nm viruses with acute, epidemic gastroenteritis is well established, but the full extent of the role of these viruses in enteric illness has been difficult to assess. Major advances in our understanding of the epidemiology of Norwalk virus were made this year using recombinant capsid protein from cloned Norwalk virus (provided to our laboratory by Dr. Estes, Baylor College of Medicine) as an antigen in an ELISA to detect serologic responses. With the availability of a relatively unlimited source of antigen, large-scale seroepidemiologic studies of Norwalk virus infection are now in progress. Preliminary serologic studies indicate that the Norwalk or a related virus is capable of infecting infants and young children more frequently than anticipated from prior limited studies. The association of such infections with illness is not known at present.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00446-09 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Testing of Rotavirus Vaccine Candidates in Venezuela and Peru

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
Instituto de Biomedicina, Caracas, Venezuela (Pérez-Schael); Instituto de Investigacion Nutricional, Lima, Peru (Lanata)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.16	0.58	0.58

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A series of successful phase I studies of the antigenicity and reactogenicity of the rhesus rotavirus-based quadrivalent vaccine candidate has allowed us to undertake a large efficacy trial of this vaccine in Venezuela. From the initial studies we learned that maximal antigenicity was attained with three doses of vaccine at a 1×10^5 PFU titer of each component, and that the vaccine was safe, although associated with mild, short-lived, febrile episodes in about 25% of the infants. The three dose schedule is being used in a placebo-controlled, double-masked, efficacy trial in Venezuela which will take place over a period of three and half years. In this "catchment" trial, the ability of the vaccine to prevent episodes of rotavirus diarrhea that are severe enough to prompt mothers to bring their children to the hospital is being evaluated. The first year of the trial has been completed; over 1100 infants have received at least the first dose of vaccine or placebo, and 61 cases of rotavirus diarrhea have been detected. Because of the double-blind nature of the study the distribution of the diarrhea cases between vaccine and placebo recipients is not available.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00451-08 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunological and Molecular Characterization of Rotavirus Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Kazuo Nishikawa, M.D.	Visiting Associate	LID, NIAID
	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1992-93

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00507-07 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Rotavirus Serotypes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Y. Green, Ph.D	Sr. Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*
University of Rochester, Rochester, NY (Madore & Dolin); University of Tampere, Tampere, Finland (Vesikari)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.84	0.17	0.67

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotavirus outer capsid proteins, VP7 and VP4, independently induce neutralizing and protective antibodes in the host. Multiple VP7 and VP4 serotypes have been identified and different combinations of serotypes of these two proteins have been found in circulating rotavirus strains. The purpose of this project is to examine the molecular basis for serotypic differences among rotaviruses and to assess the role of antigenic diversity and variation in the epidemiology of the virus. Major emphasis was placed this year on studies that examined the genetic and antigenic variation of rotavirus strains circulating during two rhesus rotavirus (RRV) vaccine efficacy trials in Rochester, New York, in 1985 and 1987. Several interesting findings emerged from this study relating to the genetic diversity among rotavirus strains circulating within the same season and those in a subsequent season in the same location.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00533-06 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Rotavirus Proteins with Monoclonal Antibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
University of Tampere, Tampere, Finland (Vesikari)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.08	0.08	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major area of research in the Epidemiology Section is the development of a rotavirus vaccine. This project was initiated with the intent of dissecting serotype-specific responses to the two major neutralization antigens following immunization and identifying laboratory correlates of resistance to rotavirus illness.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00534-06 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Non-Group A Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio (Saif); Capital Institute of Pediatrics, Beijing, China (Qian)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.08	0.08	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although the major goal of the Epidemiology Section is the development of a vaccine against Group (Gp) A rotavirus, it is important to study other antigenically distinct groups of rotavirus (B-G) since viruses belonging to two of these groups, B and C, have been implicated as etiologic agents of diarrheal disease in humans. Group B rotaviruses have been associated with large outbreaks of severe diarrhea in adults that have remained limited to China. Sporadic outbreaks and isolated cases of diarrhea caused by Group C rotavirus have been reported in several countries and this group of agents appears to be associated with illness in an older population of children (school-aged) than that of group A rotaviruses (six months to two years of age). In addition, an association between Gp C rotavirus infection and biliary atresia in infants and young children has been reported recently by others. It has been difficult to establish the epidemiology of the "non-group A" human rotaviruses because they cannot be cultivated routinely in cell culture, making the development of reagents for diagnostic tests difficult. One objective of this project is to generate tools for epidemiologic studies. However, it is also of interest to examine the molecular basis for group differences among rotaviruses because it may give insight into the mechanisms that have caused Group A rotaviruses to predominate as an enteric pathogen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00573-04 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Rotavirus Proteins in *Salmonella* Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*
University of Maryland, Baltimore, Maryland (Levine & Hone); Washington University (Curtiss); University of Missouri (Parker)

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Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.33	0.33	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of mucosal immunity in the development of resistance to many of the pathogens that have been problematic for vaccine development, including rotaviruses, is poorly understood. This area of immunologic research is relevant to the development of vaccines against mucosal pathogens because there is evidence that the delivery of antigens to the site of viral or bacterial infection is important for achieving effective immunization. The goal of this project is to assess the feasibility of a recombinant rotavirus vaccine using *Salmonella* bacteria as an antigen delivery system. In addition, we will use this system to study mucosal immune responses to rotavirus antigens.

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00574-04 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression and Distribution of Conserved and Serotype-Specific Epitopes on Rotavirus VP8

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Mitzi Sereno	Biologist	LID, NIAID
	Gisela Larralde	Guest Researcher	UCV, Venezuela

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.67	PROFESSIONAL: 0.25	OTHER: 0.42

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent studies have begun to elucidate the antigenic relationships among human rotaviruses on the basis of the antigenic specificity of the VP4 protein. A VP4 serotyping system was recently established by the neutralization technique using guinea pig antiserum to the baculovirus recombinant-expressed VP4 protein of human rotavirus (HRV) strain KU, DS-1, 1076, K8, or 69M. The criterion for serotype specificity was a greater than eight-fold reciprocal difference in neutralizing antibody titer. Four distinct VP4 serotypes were defined, namely P1, P2, P3, and P4. In addition, two P1 subtypes were identified, namely P1A and P1B.

In a recent study, the VP5 and VP8 cleavage subunits of VP4 from three different human rotavirus strains, KU (VP4 serotype 1A), DS-1 (VP4 serotype 1B), and 1076 (VP4 serotype 2), were independently expressed in *Escherichia coli*. Guinea pig antisera produced in response to immunization with these recombinant VP5 and VP8 proteins were used in cross-neutralization tests to determine the contribution of each of these two subunits to the overall antigenic specificity of VP4.

In addition, three peptides which collectively span the entire VP8 subunit of VP4 were also expressed in *E. coli*, and each peptide was analyzed by the immunoblot technique using antisera to different VP4 serotypes. These studies showed that a particular region in the VP8 extending from amino acids 84 through 180 designated (peptide B) may be involved in VP4 serotype and subtype specificities.

The amino acid sequence homology of the VP8 subunit and the B region of several human and animal rotavirus strains was compared. The actual percentage of homology was least in the B region for VP4s that were apparently identical, of the same subtype, or distinct. This was complete concordance with the VP4 classification of rotaviruses based on neutralization assays and suggest that the VP4 type can be inferred by comparing the amino acid sequence of the B region of a particular strain with that of a known VP4 type.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00576-04 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Serotype Analysis and Characterization of Rotaviruses from Malaysia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Nassar Rasool, Ph.D.	Guest Researcher	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The VP4 genetic groups of 151 field strains of human rotaviruses obtained from infants and young children with diarrhea from four locations in Malaysia, were analyzed. The strains were adapted to growth in tissue culture, and studied further by molecular hybridization of northern blotted RNA to PCR-generated probes representing amino acids 84-180 of the KU strain VP4, 83-181 of the DS-1 strain VP4, and 83-180 of both the 1076 or K8 strain VP4, representing VP4 genetic groups 1, 2, 3, and 4 (VP4 serotype 1A, 1B, 2, and 3), respectively. The majority (79%) of the field strains hybridized with the KU VP4 genetic group 1 probe and were associated with VP7 serotype 1, 3, 4, or were untypable, or of mixed VP7 serotypes. VP4 genetic group 1 (considered to belong to serotype 1A) strains were the most common in all locations in Malaysia between 1978-1988. Three strains which exhibited VP7 serotype 3 and subgroup I specificity hybridized with the K8 VP4 genetic group 4 probe. These three VP4 genetic group 4 (VP4 serotype 3) strains were detected in two different years and locations, extending the initial detection of this VP4 genetic group (the K8 strain) in Japan to a larger geographical area of Asia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00601-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Recombinant BCG-VP8 Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Mitzi Sereno	Biologist	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
MedImmune Laboratory (Stover)

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Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1992-93

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00602-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Expression of Rotavirus Outer Capsid Protein VP4 by Recombinant-Adenovirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)
Wyeth-Ayerst Research Laboratories (Davis & Selling)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1992-93

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0 864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00603-03 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Monoclonal Antibodies Directed to VP8 Subunit of VP4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Ferdinando Liprandi	Guest Researcher	IVIC, Venezuela

COOPERATING UNITS *(if any)*

UCV, Venezuela (Larralde)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.08	0.08	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously, antisera against recombinant outer capsid rotavirus VP4 proteins were used to study the VP4 antigenic polymorphism among human rotavirus strains. Four distinct serotypes and one subtype of the VP4 protein have been identified among 18 human rotavirus strains that exhibited six different VP7 serotypes. Results from cross-immunoprecipitation and neutralization assays with VP8 antisera suggest that the VP8 cleavage subunit of VP4 contains the major antigenic sites responsible for the serotype-specific neutralization of rotavirus via VP4. However, a fine map of the VP8 epitopes has been obtained only in animal rotavirus strains. These studies have been hindered in human rotaviruses by the lack of type-specific MAbs directed against the VP8 subunit.

Immunization of mice with the VP4 or VP8 cleavage subunit of VP4 of the human rotavirus KU, DS-1, 1076, K8, or 69M strain induced polyclonal antibodies that neutralized the rotavirus from which the VP4 subunit was derived. However, most of the VP4-monoclonal antibodies obtained so far have recognized heterologous strains.

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00604-03 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cold-Adaptation of Human Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
National Institutes of Health, Tokyo, Japan (Matsuno); DynCorp (Potash)

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SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.42	0.42	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two human rotavirus cell-culture isolates (strains D [VP4:1A; VP7:1], and DS-1 [VP4:1B; VP7:2]) as well as two human x human rotavirus reassortants (Wa x P [VP4:1A; VP7:3] and Wa x DS-1 [VP4:1A; VP7:2]) have been successfully adapted to grow in primary African Green Monkey kidney cells at the suboptimal temperature of 30°C, 28°C, or 26°C. These rotavirus strains were cold-adapted to determine whether cold-adapted (*ca*) mutants selected by this strategy are attenuated and suitable for use in a live virus vaccine. The 26°C, 28°C, and 30°C *ca* mutants of the D, DS-1, Wa x P, and Wa x DS-1 viruses have been plaque-purified and their phenotypes have been characterized.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00605-03 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Infection of Chimpanzees by Human Rotavirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Eileen N. Ostlund, D.V.M.	IRTA Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

Georgetown University/Twinbrook (London); Bioqual, Inc., Rockville, MD (Bradbury)

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SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<1/12	<1/12	<1/12

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are currently evaluating a rotavirus vaccination strategy in infants and young children that involves the use of an animal rotavirus strain of simian rhesus rotavirus origin in combination with three human rotavirus-rhesus rotavirus reassortant strains to form a quadrivalent vaccine. This approach which relies on the induction of antibody to the rotavirus outer capsid protein VP7 was adopted in an attempt to achieve protection against each of the four medically important VP7 rotavirus serotypes. If the desired level of protection is not achieved by this vaccine, we are considering two major alternative strategies: (i) the use of one or more cold-adapted human rotavirus mutants which would possess the human rotavirus VP4 (whereas the quadrivalent vaccine possesses the rhesus rotavirus VP4), an important outer capsid protein that also induces neutralizing antibodies and may be important in the induction of heterotypic immunity; and (ii) replacement of a single gene of a human rotavirus strain or of a human rotavirus x human rotavirus reassortant, that is associated with virulence with that of a bovine rotavirus strain, thus preserving the VP4 and VP7 specificities of the human rotavirus strain. It is important to identify an experimental animal that develops diarrhea following oral administration of a virulent human rotavirus strain in order to determine if either of these approaches yield viruses that are attenuated. Previously, we induced rotavirus diarrhea, and virus shedding and a serologic response to rotavirus in one of two chimpanzees that were given a virulent VP7 serotype human rotavirus strain by the alimentary route. This year we administered a cold-adapted (26°C) human rotavirus reassortant that possesses ten genes (including VP4) from human strain Wa and one gene encoding VP7 from human strain DS-1 (a VP7 serotype 2 strain). The chimpanzee did not develop illness or shed rotavirus. Serologic studies are pending.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00639-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Epidemiology of Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
Medical University of Southern Africa (Steele); Institute of Virology, University of Pavia, Pavia, Italy (Gerna); Akita University School of Medicine, Akita, Japan (Nakagomi)

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Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.25	PROFESSIONAL: 0.33	OTHER: 0.92

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The molecular epidemiology of human and animal rotaviruses has been investigated by hybridization and sequencing techniques with four major objectives: (i) investigate the role of the viral outer capsid protein VP4 in virulence of human rotaviruses; (ii) develop hybridization probes specific for the genes encoding the rotavirus proteins VP4 and VP7 (the two outer capsid proteins involved in viral neutralization) to be used in the identification of rotavirus serotypes and delineation of their distribution; (iii) identify and characterize new VP4 and VP7 serotypes of human and animal rotavirus strains; and (iv) determine the relative proportion of the neutralization antibody response to natural rotavirus infection that is specific for each of the outer capsid proteins, VP4 and VP7. Considerable progress has been made in each of these areas of research.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00640-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthetic Analogs of Rotavirus Genomic RNA Expressing a Foreign Marker Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	An-Dao Yang, M.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*

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SECTION
Epidemiology Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.58	0.67

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The rotavirus genome consists of 11 segments of double-stranded (ds) RNA of 667 (segment 11) to 3,302 bp (segment 1). The 5' and 3' ends of rotavirus RNAs contain distinct, unrelated terminal consensus sequences of 7 to 10nt which are assumed to be important *cis*-acting signals. These presumably include, at the 3' ends of plus and minus strands, the viral promoters for the synthesis of the complementary strand. The termini also might contain sequences important for packaging and for regulation of expression at the levels of transcription, replication and translation. The 5' end of the plus strand has a methylated cap structure and neither strand is polyadenylated. cDNAs were constructed to encode three single-stranded, plus-sense analogs of rotavirus gene segments containing the chloramphenicol acetyl transferase (CAT) translational open reading frame (orf) as a reporter for expression. One analog, which has been previously described, contained the CAT orf flanked by the 5'- and 3'-terminal non-coding regions of genome segment 9 of the porcine rotavirus strain OSU. To test the effects of substituting viral non-coding sequences from a different genome segment, a second analog contained the CAT gene flanked by the non-coding regions of genome 11 of the simian rotavirus SA-11. In addition, to determine whether the viral orfs which had been replaced by the CAT orf might contain *cis*-acting signals, a third analog consisted of strain OSU genome segment 9 in its entirety with the CAT orf inserted following position 44. Transfection of these analogs individually into 293 cells infected with rotavirus strain OSU resulted in the efficient expression of CAT. Furthermore, each of the three CAT-containing RNA analogs was packaged into a form that could transmit CAT expression to fresh cells for at least two serial passages. Passage of CAT expression was insensitive to ribonuclease but was neutralized by guinea pig antiserum raised against strain OSU, suggesting that the analog was packaged within virus particles. The amount of CAT expression following passage was much less than that of the initial transfection. The three different RNA analogs were essentially indistinguishable in their efficiencies of CAT expression and passage. This supports the idea that the *cis*-acting sequences required for all aspects of the rotavirus replicative cycle are located in the non-coding regions and are not augmented by the inclusion of additional viral sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00641-02 LID

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Neutralizing Epitopes on Rotavirus Outer Capsid Proteins (VP4 and VP7)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.16	0.008	0.008

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two serotype-specific and two serotype-cross-reactive neutralizing monoclonal antibodies (N-mAbs) were utilized to study the antigenic sites involved in the formation of neutralization epitopes. Single, double, or triple neutralization-resistant mutants were selected by using the N-mAbs sequentially *in vitro*. Nucleotide and deduced amino acid (*aa*) substitutions found on the VP7 of such mutants showed that: (i) in addition to variable regions VR-5 (*aa* 87-100), VR-7 (*aa* 141-150), VR-8 (*aa* 208-224), and VR-9 (*aa* 235-242), *aa* 290 and 291 in the constant region of the VP7 are involved in neutralization, and (ii) VR-5, VR-7, VR-8, VR-9, and *aa* 290 and 291 are functionally related to one another. In order to further analyze neutralization sites on the VP7 and to study possible synergistic or antagonistic effects among the N-mAbs employed in this study, antigenic variants were generated in the presence of three N-mAbs. Nucleotide sequence analysis of the VP7-encoding gene of the mutants is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00642-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Norwalk and Norwalk-Like Viruses (27nm viruses)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Judy F. Lew, M.D.	Sr. Research Investigator	LID, NIAID
Others:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*
Baylor College of Medicine, Houston, Texas (Estes)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.08	1.0	0.08

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Norwalk and Norwalk-like viruses have been shown to be the most common cause of epidemic acute nonbacterial gastroenteritis, but because of the fastidious nature of these agents, little is known about the molecular epidemiology of these 27 nm viruses. Our goal is to determine the molecular characteristics that are important in the epidemiology, immunogenicity, and pathogenesis of these agents. Towards this goal, we have sequenced: (i) approximately 2800 consecutive bases from the genome of a 27 nm Norwalk-like virus obtained from a serviceman deployed to the Middle East during Desert Shield, as well as a 27 nm virus obtained from a child in Toronto, Canada; (ii) approximately one-third of the proposed capsid region of three other Toronto virus strains; (iii) a portion of the polymerase region of two Desert Shield viruses, several other Toronto viruses, and a virus from Norfolk, Virginia. In addition, we have cloned the entire proposed capsid region of the Norwalk virus, a Desert Shield virus (DS395), and a Toronto virus (TV4) for use in protein expression studies.

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00643-02 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Rotavirus Outer Capsid Protein VP4 by a Baculovirus Recombinant

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Baoguang Li, Ph.D.	Guest Researcher	LID, NIAID

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.08	0.08	0

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotavirus is the most important cause of severe viral gastroenteritis in humans and in animal species. The development of an effective vaccine requires not only knowledge of the antigens involved in protection but also an understanding of the antigenic variation of a particular antigen. Serotype specificity has been largely defined by VP7, a glycoprotein, which is one of the two major neutralization proteins located on the outer capsid and encoded by RNA segments 7, 8, or 9 (depending on the virus strain). Altogether, 14 rotavirus VP7 (G) serotypes have been identified, 9 among human rotaviruses (HRVs).

The other outer capsid protein VP4 can also independently mediate active and passive immunity. VP4 constitutes the viral outer capsid spike protein and is encoded by gene segment 4. An additional rotavirus serotyping system based on VP4 has been proposed based on nucleic acid hybridization, sequence analysis, and cross-neutralization results. In a recent study, four different VP4 alleles present on HRV strains KU (genetic group 1), DS-1 (genetic group 2), 1076 (genetic group 3), or K8 (genetic group 4) were separately expressed in *Spodoptera frugiperda* (sfs) cells using the baculovirus vector *Autographa californica* nuclear polyhedrosis virus. Antisera produced in response to immunization with these recombinant VP4 proteins allowed us to define the extent and distribution of VP4 antigenic polymorphism among rotaviruses currently circulating in humans. Four distinct VP4 serotypes and one subtype (P1A, P1B, P2, P3, and P4) were identified among human rotavirus strains belonging to VP7 serotype 1, 2, 3, 4, or 8. The P1A, P1B, P2, and P3 serotypes correspond to VP4 genetic groups 1, 2, 3, and 4, respectively. In this study, we investigated the antigenic relationship of the VP4 of HRV strain 69M (genetic group 5). In two-way neutralization assays, there was no serological relationship between rotavirus 69M and previously described P serotypes. It thus appears that the 69M strain is a distinct VP4 subtype and should be classified as VP4 serotype 4.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00684-01 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Serological Characterization of Equine Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eileen Ostlund, D.V.M., Ph.D.	IRTA Fellow	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

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Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<1/12	<1/12	0

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two rotavirus outer capsid proteins, VP4 and VP7, are the major antigens targeted by neutralizing antibody. Both outer capsid proteins are involved in virus virulence. Although VP7 serotypes are well characterized, little is known about the antigenic relationships among VP4s of many animal rotavirus strains. In order to examine the VP4 serotypes of equine rotaviruses, hyperimmune sera induced by VP4 antigens representing three prototype equine strains, H1, H2, and L338 will be prepared and used in serological characterization of equine rotavirus strains. Toward this end, reassortants were generated between three equine rotavirus strains, H1 [VP7:3], H2 [VP7:5], or L338 [VP7:13] and a human rotavirus strain DS-1 (7729) [VP4:1A;VP7:2]. Plaque-purified reassortants containing the equine genomic segment for VP4 in the background of DS-1 VP7 are being prepared as immunogens.

Cold-adaptation of rotaviruses may be associated with attenuation of virulence. In order to investigate the genetic basis for cold-adaptation, propagation of equine rotavirus H2 at progressively lower temperatures was achieved starting with routine culture at 37 C and progressing downward in temperature to growth at 28 C. The H2 virus adapted to grow at 30 C was plaque purified at that temperature. Sequence analysis of viruses plaque purified at different stages in the cold-adaptation process will permit analysis of specific mutations that were generated and that may confer attenuation of virulence.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00685-01 LID

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Studies of the Cold-Adapted Human Rotavirus Phenotype

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eileen Ostlund, D.V.M., Ph.D.	IRTA Fellow	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<1/12	<1/12	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of specific rotavirus genes involved in conferring phenotypes characterized by either: (i) cold-adaptation (26°C), or (ii) ability to grow at 39°C (non-temperature-sensitive) is being investigated.

Reassortants of cold-adapted (26°C), temperature-sensitive human rotavirus Wa x P with wild-type human rotavirus DS-1 (non-cold-adapted, non-temperature-sensitive) were generated and selected, without antibody pressure, at 30°C or 39°C in AGMK or MA104 cells. Reassortants were identified by PAGE of genomic RNA and were plaque purified at the respective temperatures. Rotavirus reassortants containing the Wa x P (*ca*) or DS-1 parental genes for VP6 and VP7 were also confirmed by ELISA. Rotavirus gene segments from the Wa x P (*ca*) parental strain which were present in reassortants able to grow at 39°C include genes 7, 8, 9 [VP7] and 11. Reassortants containing DS-1 genes 4 [VP4], 6, 7, 9, and 11 were able to maintain the *ca* phenotype.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

LABORATORY OF IMMUNOGENETICS

ANNUAL REPORT

October 1, 1992 to September 30, 1993

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LABORATORY OF IMMUNOGENETICS

National Institute of Allergy and Infectious Diseases
October 1, 1992 to September 30, 1993

RESEARCH PROGRESS

Studies in the Laboratory of Immunogenetics emphasize genes involved in immune function and molecular mechanisms involved in responses to immunization and infection. Genetic mapping studies of the human TCR gene complex describe sequences for genetically determined regions of insertion/ deletion and show that this area encodes functional genes. Progress in construction of a physical map of the rabbit MHC includes description of a 400 kb genomic fragment which includes all class III genes. Experiments involving infection of the rabbit with the human retrovirus, HTLV-I, suggests mechanisms by which HTLV-I causes disease in certain individuals but not in others. In studies of HIV-1 infection in rabbits, virus infected blood cells have been passaged for several years in rabbits; receipt of infected blood has resulted in an immune deficiency characterized by low levels of CD4 T cells. Molecular dissection of antigen processing has shed new light on the early cellular events crucial to mounting an immune response. Studies on natural killer cells indicate that a novel cell surface molecule may be involved in directing their cytolytic activity. Studies on T-cells in the mouse that have suppressor or activator activity *in vivo* indicate a role for lymphokines.

Studies of the Human T-cell Receptor Complex

Studies concerning the polymorphism of genes in the human TCR complex and the role of gene variation in normal immune responses and disease susceptibility have advanced in the past year. Progress toward a definitive map of the TCR genes was made showing that genetic variation in the TCR β gene complex serves to both expand and limit the TCR repertoire. A region of genetically determined insertion/deletion (IDRP) in the V gene complex contains TCR genes that are unique to inserted haplotypes including a previously uncharacterized member of the Vβ7 family, a pseudogene in the Vβ9 family, and a Vβ13 gene that is a precise replicate of a Vβ13 gene encoded at another location within the complex and present in deleted TCRβ haplotypes. Recent mapping data further indicate that certain V genes are not encoded within the TCR complex on chromosome 7. An additional cluster of Vβ genes was localized to chromosome 9 by *in situ* hybridization and by use of a somatic cell hybrid panel. Analyses of families and of single sperm revealed that genetic recombination occurs at a considerably higher frequency within the 5' end of the TCR β gene complex than would be expected assuming average recombination rates. This observation provides an explanation for the extensive heterogeneity observed in the combinations of markers in this region and indicate approaches for TCR and disease studies. Findings that polymorphism includes presence or absence of certain TCR genes and that certain Vβ genes are not within the complex have important implications to arguments about TCR gene expression in normal responses or in various autoimmune states. (**Robinson, Day, Zhao,**).

Quantitative studies of TCR V gene expression have shown that TCRβ gene usage is not proportionate to the number of V genes present in the genome. The extent of the TCR repertoire was investigated by analysis of genomic and cDNA libraries prepared from lymphocytes of members of families well characterized for HLA. These studies revealed both extensions and limitations of TCR Vα and Vβ repertoires. The known repertoire has been extended by identification of new Vα and Vβ families, new members of defined families and by descriptions of allelic polymorphism in V gene segments. Limitations of TCR repertoires are imposed by the fact that certain V genes have been characterized as pseudogenes. The pseudogenes include allelic variants of Vβ12.X and Vβ20 that have nucleotide substitution resulting in a stop codon and thus are non-functional alleles, and two alleles of Vβ6.1 that are pseudogenes due to the replacement of a highly conserved amino acid. Holes in the TCR repertoire caused by these unexpressed TCR alleles appear to contribute to susceptibility to a number of clinical subsets of JRA. In another autoimmune disease, Reiter's Syndrome, allelic variation in genes encoded within the HLA Class II region may contribute to susceptibility to disease. TAP genes are thought to function as transporters of peptides for presentation by Class I molecules and may contribute to susceptibility to disease by influencing the selection of peptides. The frequency of the TAP2A allele was increased in the patient group as compared to control groups and all patients were TAP2A+. Certain HLA-DQ alleles were over-represented in the patient group as well. Detailed knowledge of the extent and diversity of the germline TCR repertoire will greatly facilitate our ability to understand the role of TCR genes in immune responses in normal and disease states. **(Robinson, Barron, Deulofeut)**

Studies of the Rabbit MHC

Investigations of the major histocompatibility complex of the rabbit (RLA) have provided further evidence concerning the similarities and differences between RLA and the well characterized MHC regions of human and mouse. A gene encoding the DO beta chain was cloned from rabbit spleen cDNA and its expression shown to be confined to mature B cells and to thymus (**Chouchane**). The gene was studied in 8 different haplotypes and the DOB coding region was completely conserved in each. Polymorphism was detected by use of restriction enzymes indicating that flanking sequences not as highly conserved as the coding regions. The gene encoding the alpha chain partner of DOB is being sought using probes for the human counterpart. The rabbit should provide an excellent model system in which to study the function of this highly conserved class II molecule.

Efforts to construct a complete physical map of the rabbit MHC have progressed. Molecular genetic analyses of the rabbit MHC (RLA) using pulsed field gel electrophoresis have shown that the relative order of class II genes (DP, DO, DQ, DR) is identical to that in humans and similar to that in mouse. However, a major difference from either HLA or H-2 was observed at the DR end of the RLA class II complex where class I genes are located in close proximity to DR with no interposed class III sequences. Recently a 380 kb stretch containing class III genes, including those for complement components and for heat shock protein, has been mapped (**Ismail**). No class I or class II genes have been linked to this class III cluster by pulsed field electrophoresis, although breeding studies reveal linkage of class III to class I and II genes. A probe for the TNFa gene, which lies between class I and class III in human and mouse, has been prepared and will be used in an attempt to order the various MHC regions in the rabbit.

HTLV-Infection in Rabbits

Human T cell lymphotropic virus-I (HTLV-I) is a retrovirus which is the etiologic agent of human adult T cell leukemia and the neurologic disorder termed Tsp/Ham. Although these and various other human disease conditions have been associated with HTLV-I infection the majority of HTLV-I infected individuals remain asymptomatic. Although hypotheses concerning the progression of HTLV-I infection to disease have been advanced, no clear explanation for the myriad activities of this virus is available. Structural studies of HTLV-I reveal a highly conserved sequence for isolates taken from diverse populations and from individuals with different disease manifestations. As opposed to HIV and SIV sequences which vary enormously, HTLV-I sequences seldom diverge more than several percent. No infectious molecular clones of HTLV-I have been reported, therefore it is not yet possible to relate biologic activity to the observed structural variation. Although the rabbit can be readily infected with HTLV-I, the utility of the rabbit infection has been limited by the fact that, as in human infection, HTLV-I only causes disease in rare instances.

A series of HTLV-I cell lines were prepared by in vitro transformation of rabbit peripheral blood mononuclear cells (PBMC) using HTLV-I cell lines of human or rabbit origin as virus source (**Sawasdikosol**). The majority of HTLV-I lines obtained, as well as the human or rabbit progenitors of these lines, mediated no disease in injected animals. Seroconversion and chronic cell infection were the only consequences even in rabbits injected with as many as 5 x 10^8 live HTLV-I infected cells. By contrast, certain infected lines killed rabbits in a dose dependent manner; animals injected with the lethal lines developed high temperature within four days of injection and usually died by day 8. A detailed description of organs from the affected animals is in progress; it appears that the HTLV-I lines cause infiltration of lung, lymph nodes, spleen and liver in a manner similar to adult T cell leukemia lymphoma in humans. Injection of irradiated cells was not lethal within the same short time frame as live cells, but most hallmarks of the disease were present (**Simpson**). The majority of rabbit HTLV-I cell lines including several derived in the same fashion as the lethal line cause no overt disease. A detailed study of this phenomenon is underway; results here can shed light on why HTLV-I infection in humans causes serious disease in only a small percentage of infected individuals whereas most infected persons remain asymptomatic. Progress in comparing lethal and nonlethal cell lines includes a) structure of virus in lethal and non lethal cell lines (**Zhao, Robinson**); b) Characterization of the lines as CD4-, CD8-, $\gamma\delta$ TCR T cells c) disease caused by lethal lines has been shown to be similar to human adult T cell leukemia/ lymphoma; d) the lethal HTLV-I cell line causes apoptosis in rabbit PBMC, whereas a non lethal line causes cell proliferation (**Leno**). Research efforts to find differences between lethal and non lethal cell lines concentrate on host responses to the cell lines and the viruses produced by them. Their are preliminary indications that antibodies to members of the heat shock protein families are important to survival of challenge with HTLV-I.

Infection of Rabbits with HIV-1

It was shown previously that laboratory rabbits may be infected with HIV-1 by administration of a single dose of infected human T cells. Infection in rabbits injected with HIV-1 has been detected by seroconversion, detection of virus in cells and organs using PCR and *in situ* hybridization, and by isolation of virus from PBMC. While the fact that rabbits can be infected with HIV is promising, the slow course of infection, difficulty in isolation of virus and the absence of consistent disease signs in infected rabbits have limited utility of this model for AIDS. Information gained in recent efforts to improve the model suggest that infection in the rabbit may be enhanced in several ways. Adaptation of HIV to grow more efficiently in rabbits has been attempted by long term *in vivo* passage; injection of as little as 1 ml of blood from animals receiving splenocytes from a rabbit infected with HIV-1 and held for 2.3 years has resulted in lymphopenia, CD4 T cell depletion and for 2 of 12 rabbits, death (**Simpson**). Initial results from structural analyses of HIV-1 amplified from a spleen cell recipient shows nucleotide sequence identity of 60-85% to the original input isolate, HIV_{Lai} (**Hague**). Compared to HIV-1, HTLV-I infects rabbit cells with high efficiency. Comparison of Northern Blots from infected rabbit cells indicated that HIV-1 transcripts are relatively unstable, while those of HTLV-1 are as stable as endogenous message (**Cho**). CAT constructs utilizing the 5' or 3' LTRS from HIV-1 or HTLV-I to provide the promoter or 3' processing signals are being used to compare LTR-mediated gene expression in rabbit and human cells. Data from these expression studies will be used to guide the construction of HIV-1 clones containing alternative LTR sequences.

Rabbit CD4 and HIV Infectivity *in vitro*

The gene for rabbit CD4 (RbCD4) marker was cloned and was used to verify the specificity of a McAb directed against CD4, this has been used to establish normal values for this marker and to monitor rabbits infected with HIV. Significant depletion of CD4 was seen in some infected rabbits infected by passage of blood from other infected rabbits. In addition the CD4 gene was used in transfection experiments to determine its efficacy as a receptor for HIV-I. RbCD4 transfectants of the human cell line, Raji, were compared with this line expressing human CD4 (**Hague**). The RbCD4 proved to be poor receptor and therefore studies of rabbits transgenic with human CD4 were given priority. Animals have been obtained and shown to express the transgene for HuCD4 on appropriate T cells. Infections of PBMC from the transgenics with various strains of HIV are now underway. Other markers for rabbit lymphoid cell populations were studied and genes encoding them cloned; these include the T cell receptors δ and γ, CD8β and IL-2rα. It was observed using reagents derived in our studies that all HTLV-I transformed cell lines derived by infection of rabbit PBMC with virus in vitro had a similar phenotype (**Sawasdikosol**). All lines were CD4-, CD8-, and TCR δγ. Morphological examination using scanning electron microscopy revealed these to be similar to human γδ cells. $CD4^+$ and $CD8^+$ lines were derived from PBMC of HTLV-I infected rabbits and $CD4^+$ TCR αβ HTLV-I lines were derived from rabbit thymus eliminating the possibility that the HTLV-I isolates used here transform only $CD4^-CD8^-$ TCR δγ cells. The percentage of δγ cells in rabbit PBMC is relatively high (23% in adult rabbits); this with diminution of $CD4^+$ and $CD8^+$ cells in IL-2 supplemented PBMC or thymocyte cultures may account for selection of rabbit HTLV-I infected δγ T-cell lines *in vitro*. The availability of well characterized T cell lines with diverse *in vivo* effects in the rabbit HTLV-I disease model allows evaluation of roles played by cell type in HTLV-I mediated disease.

Molecular Mechanisms of Antigen Presentation

Most immune responses occur only if a T lymphocyte recognizes foreign antigen in association with a self class II molecule of the major histocompatibility complex (MHC). Antigen must be processed for presentation by class II molecules and this processing generally involves endocytosis of antigen into an acidic compartment where newly synthesized class II molecules have been transported. The foreign peptide/MHC class II complexes recognized by CD4 T cells are thus formed intracellularly. The aim of this project is to define the potential sources of antigen for presentation by class II molecules, and to determine how class II molecules are transported to antigen-processing compartments. A genetic approach was used to demonstrate that several processing pathways exist for antigen presentation by class II molecules. The classical pathway for presentation of exogenous antigen requires newly synthesized class II molecules and a function encoded by an unknown gene within the class II region of the MHC. However, presentation of the influenza virus hemagglutinin antigen was independent of protein synthesis and of the function encoded by the unknown gene, implying that two processing pathways exist. In addition, it was demonstrated that two endogenous processing pathways lead to the presentation of cytosolic antigen to class II-restricted T cells, one is dependent on the transporter for antigen presentation (TAP), the other is not. In addition, the TAP-independent pathway requires another function that maps within the class II region of the MHC. This previously unsuspected presentation of cytosolic proteins by class II molecules has important implications for T cell repertoire selection, T cell tolerance, autoimmunity and vaccine design (**Malnati, Long, Pinet, Guéguen, Rojo**). Targeting of newly synthesized class II molecules to endosomes is mediated by the invariant chain (Ii), but the intracellular transport route is not known. This project demonstrated that a large population of class II-Ii complexes reach endosomes by rapid internalization from the cell surface. The highly efficient endocytosis was mediated by the cytoplasmic tail of Ii. Following internalization, Ii dissociated from class II-Ii complexes, suggesting that this pathway represent an important mechanism for loading class II molecules with peptides from several endocytic compartments (**Roche, Warmerdam**).

NK Cell Specificity

Natural Killer (NK) cells are thought to play a role in the control of viral infection before the establishment of a specific cytolytic T cell response mediated by CD3-positive MHC-restricted T cells. Recently, specific recognition of alloantigens by NK cells was reported. Most of the cells displaying NK activity belong to the $CD3^-$ $CD16^+CD56^+$ subset of peripheral blood lymphocytes. The mechanism of target cell recognition by NK cells is still unknown. The aim of this study was (i) to determine whether target cell elements control the recognition and lysis by NK cells, and (ii) to purify a surface molecule from NK cells in sufficient amount to obtain amino-acid sequences. As a first step, it was established that NK clones from a normal individual were able to specifically recognize and kill normal cells from the same individual that had been infected in vitro with human Herpesvirus-6 (HHV-6). The results showed for the first time that specificity in target cell recognition by NK cells is controlled at several levels: first, at the level of the NK clone itself, and second, by genetically variable elements on the target cells that restrict recognition by NK cells. By use of a monoclonal antibody, a NK cell surface protein that is involved in target cell recognition was purified and partial amino-acid sequences were obtained. The present goal of this project is to obtain molecular clones for this molecule and to define its role in target cell recognition.

(**Malnati, Wagtmann**).

Regulation of the Antibody Response to Microbial Polysaccharide Antigens

Prior treatment with small amounts of inner core-region polysaccharides, from different gram negative bacteria, induced profound T cell mediated suppression of the antibody response. Such suppression is due to an expansion of the pool of suppressor T cells (Ts) activated during the course of a normal antibody response to most bacterial polysaccharide antigens. The minimal structure require to induce suppression is a hexa-saccharide composed of glucose, 2 heptoses and 3 KDO residues. Since this structure is conserved among gram negative bacteria, it may represent an important virulence factor, especially when associated with lipid A molecules, e.g. those present in Pseudomonas species, that are unable to abolish the expression of Ts function. (**Taylor, Baker**).

Treatment with lipid A or monophosphoryl lipid A abolishes the expression of suppressor T cell (Ts) function, without adversely influencing the expression of other T cell activity. This is due to the number and location of fatty acyl groups present, as well as to fatty acyl chain length. The inability of the lipid A of Pseudomonas aeruginosa to abolish Ts function, in conjunction with the capacity of inner-core region polysaccharides of its lipopolysaccharide to stimulate Ts activity suggest an important virulence mechanism (**Hraba, Baker**).

LABORATORY OF IMMUNOGENETICS
ANNUAL REPORT
October 1, 1992 to September 30, 1993

HONORS AND AWARDS

Dr. Thomas Kindt, Chief of the Laboratory of Immunogenetics, presented invited seminars at University of California at San Francisco, at TSI Inc., at Haverford College and for the FAES course in clinical immunology. Laboratory data were presented at the meeting of the Laboratory of Tumor Cell Biology, at the conference for Science Innovation and at a FASEB summer conference. Dr. Kindt served on a delegation from the Fogarty International Center and the NIH to visit and prepare a report on research activities in laboratories in and near Mexico City. Dr. Kindt has been appointed to the publication committee of the American Association of Immunologists and is an Associate Editor of the *FASEB Journal* and North American regional editor of *Research in Immunology.* Dr. Kindt serves on the Board of Scientific Visitors for the Oklahoma Medical Research Foundation in Oklahoma City and serves in a similar capacity for the Research Program at the Virginia Mason Institute in Seattle, Washington. Dr. Kindt continues to serve on the NIH Allergy and Immunology Study Section and on the scientific advisory boards of Oncor Inc., Gaithersburg MD, and Innovir Laboratories Inc., New York, NY.

Dr. Mary Ann Robinson was an invited speaker at the British Society of Immunology Autumn meeting in London, for the FDA, and for the Inter-Institute Genetics Program. She served on the Scientific Affairs Committee for the American Society of Histocompatibility and Immunogenetics (ASHI) and was a member of the Abstract Review comittee and chaired a session for the ASHI 1992 annual meeting. She continues to serve as a member of the editorial board of *Human Immunology.*

Dr. Eric Long received the NIH Director's award. He was Plenary Speaker at the Meeting of the British Society for Immunology and speaker at the Immunology Seminar Series of the University of Washington, Seattle, WA. In addition, he presented at a seminar at the University of California in San Francisco and at Mount Sinai Medical Center. He also participated as a speaker at the Keystone Symposium on "Emerging Principles for Vaccine Development" Taos, NM; speaker at the Immunology Seminar Series of the Massachusetts General Hospital, Boston, MA; and at the Immunology Seminar Series of the University of Chicago, Chicago, IL.

Dr. Phillip Baker presented lectures and seminars at several universities and medical schools. He is on the Editorial Board of the AMS News, serves on the Public Relations Committee of the American Society for Microbiology (ASM) and also serves on the Publications Board of the International Endotoxin Society. He was elected Councillor and appointed to the Board of Governors of the International Endotoxin Society.

Dr. Christopher Taylor was invited to convene a Divisional Symposium on "Cytokines and Microbial Infections" at the annual meeting of the American Society for Microbiology in Atlanta. As chairman of the Immunology Division of the ASM, he organized six seminars including one on "Genetic Immunization: Implications for the Technology". He also gave a talk during NIAIDs "Introduction to Biomedical Research Program" in February 1993. In addition, he presented seminars at medical colleges and reviewed manuscripts for Immunology and Infectious Diseases.

LABORATORY OF IMMUNOGENETICS

ANNUAL REPORT

October 1, 1991 to September 30, 1992

ADMINISTRATIVE REPORT

The Laboratory of Immunogenetics occupies laboratory and animal space in the NIAID Twinbrook II facility in Rockville, Maryland. Laboratories and office spaces in the building are in process of renovation and expected to be fully operational in the Fall of 1993. The laboratory continues to maintain a contract facility at Spring Valley Laboratories for animals infected with human retroviruses. In the past year the Immunogenetics Research Section of the laboratory was joined by Dr. Harold Deulofeut from the Dana Farber Institute at Harvard; he will work with Dr. Mary Ann Robinson. Dr. Camilla Day left the section to take a position in the NIH division of grants and contracts. The Molecular Immunology Section was joined by Dr. Petronella Warmerdam from State University Utrecht in The Netherlands and by Dr. Susanna Rojo from Foundation Jimenez Diaz in Madrid, Spain both of whom will work with Dr. Eric Long. The Microbiology and Immunology section of LIG will be terminated July 1993. Dr. Baker will assume a position in the Extramural Branch of NIAID.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00143-23

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Control of the Antibody Response to Microbial Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.J. Baker, Ph.D.	Head, Microbiology & Immunology Section, LIG, NIAID
Other:	C.E. Taylor, Ph.D.	Senior Staff Fellow, LIG, NIAID
	T. Hraba, Ph.D.	Visiting Scientist, LIG, NIAID -

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Microbiology and Immunology

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PROJECT INACTIVE

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00144-29

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of the Antibody Response to Microbial Polysaccharides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.J. Baker	Head, Microbiology & Immunology Section, LIG, NIAID
Other:	C.E. Taylor	Senior Staff Fellow, LIG, NIAID
	T. Hraba	Visiting Scientist, LIG, NIAID
	P.W. Stashak	Microbiologist, LIG, NIAID
	M.B. Fauntleroy	Biologist, LIG, NIAID

COOPERATING UNITS *(if any)*

* K. Takayama, Mycobacteriology Lab., WI; ** E. Rietschel, U. Zahringer, Borstel Institut für Experimentelle Biologie und Medizin, Germany

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prior treatment with small amounts of inner core-region polysaccharides, from different gram negative bacteria, induced profound T cell mediated suppression of the antibody response. Such suppression is due to an expansion of the pool of suppressor T cells (Ts) activated during the course of a normal antibody response to most bacterial polysaccharide antigens. The minimal structure require to induce suppression is a hexa-saccharide composed of glucose, 2 heptoses and 3 KDO residues. Since this structure is conserved among gram negative bacteria, it may represent an important virulence factor, especially when associated with lipid A molecules, e.g. those present in Pseudomonas species, that are unable to abolish the expression of Ts function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00145-26

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	P.J. Baker	Head, Microbiology and Immunology Section, LIG, NIAID
Other:	C.E. Taylor	Senior Staff Fellow, LIG, NIAID
	T. Hraba	Visiting Scientist
	P.W. Stashak	Microbiologist, LIG, NIAID
	M.B. Fauntleroy	Biologist, LIG, NIAID

COOPERATING UNITS (*if any*)

*K. Takayama, Mycobacteriology Lab., WI; **E. Rietschell, U. Zahringer, Institut für Experimentelle Biologie und Medizin, Germany; ***G. Pevez-Pevez, Division Infectious Diseases, Dept. of Medicine, Vanderbilt Univ. School of Medicine, TN

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Treatment with lipid A or monophosphoryl lipid A abolishes the expression of suppressor T cell (Ts) function, without adversely influencing the expression of other T cell activity. This is due to the number and location of fatty acyl groups present, as well as to fatty acyl chain length. The inability of the lipid A of Pseudomonas aeruginosa to abolish Ts function, in conjunction with the capacity of inner-core region polysaccharides of its lipopolysaccharide to stimulate Ts activity suggest an important virulence mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00166-16

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characteriazation of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. J. Kindt	Chief	LIG, NIAID
Other:	L. Chouchane	Visiting Fellow	LIG, NIAID
	A. Ismail	Visiting Fellow	LIG, NIAID

COOPERATING UNITS *(if any)*
Dr. Patrice Marche, Institut Pasteur, Paris

LAB/BRANCH
Labortory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	2.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigations of the major histocompatibility complex of the rabbit (RLA) have provided further evidence concerning the similarities and differences between RLA and the well characterized MHC regions of human and mouse. A gene encoding the DO beta chain was cloned and its expression shown to be confined to mature B cells and to thymus. The gene was studied in 8 different haplotypes and coding regions conserved in each, polymorphism was however detected by use of restriction enzymes indicating that flanking sequences not highly conserved. The gene encoding the alpha chain partner of DOB is being sought using probes for the human counterpart. The rabbit should provide an excellent model system in which to study the function of this highly conserved class II molecule. Efforts to construct a complete physical map of the rabbit MHC have progressed. A 380 kb stretch containing class III genes, including those for complement components and for heat shock protein, has been mapped. No class I or class II genes have been linked to this class III cluster by pulsed field electrophoresis, although breeding studies reveal linkage of class III to class I and II genes. A probe for the TNFa gene, which lies between class I and class III in human and mouse, has been prepared and will be used in an attempt to order the various MHC regions in the rabbit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00168-16

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Surface Markers of Rabbit Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.J. Kindt	Chief	LIG, NIAID
OTHER:	Bishop Hague	Staff Fellow	LIG, NIAID
	Sansana Sawasdikosol	Biologist	LIG, NIAID

COOPERATING UNITS (if any)

Dr. B. Snyder, Dr. S. Wadsworth, Dr. H. Rudolph, TSI Inc., Worcester, MA

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Summary of Work

Studies of cell surface markers for rabbit lymphoid cell populations have continued and progress has been made in several areas of interest. The gene for rabbit CD4 (RbCD4) marker was cloned and was used to verify the specificity of a McAb directed CD4, this has been used to establish normal values for this marker and to monitor rabbits infected with HIV. Significant depletion of CD4 was seen in some infected rabbits. In addition the CD4 gene was used in transfection experiments to determine its efficacy as a receptor for HIV-I. RbCD4 transfectants of the human cell line Raji were compared with this line expressing human CD4. The RbCD4 proved to be poor receptor for HIV-1 and therefore studies of rabbits transgenic with human CD4 have been given priority. Animals have been obtained and shown to express the transgene for HuCD4 on appropriate T cells. Infections of PBMC from the transgenics with various strains of HIV are now underway. Other markers for rabbit lymphoid cell populations were studied and genes encoding them cloned; these include the T cell receptors δ and γ, CD8β and IL-2rα. It was observed using reagents derived in our studies that all HTLV-I transformed cell lines derived by infection of rabbit PBMC with virus in vitro had a similar phenotype. All were CD4$^-$, CD8$^-$, and TCR δγ. Morphological examination using scanning electron microscopy revealed these to be similar to human γδ cells. CD4$^+$ and CD8$^+$ lines were derived from PBMC of HTLV-I infected rabbits and CD4$^+$ TCR αβ HTLV-I lines were derived from rabbit thymus eliminating the possibility that the HTLV-I isolates used here transform only CD4$^-$CD8$^-$ TCR δγ cells. The percentage of δγ cells in rabbit PBMC is relatively high (23% in adult rabbits); this with diminution of CD4$^+$ and CD8$^+$ cells in IL-2 supplemented PBMC or thymocyte cultures may account for selection of rabbit HTLV-I infected δγ T-cell lines *in vitro*. The availability of well characterized T cell lines with diverse *in vivo* effects in the rabbit HTLV-I disease model allows evaluation of roles played by cell type in HTLV-I mediated disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00170-16

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Functional Analysis of Human Class II Histocompatibility Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eric O. Long, Ph.D	Visiting Scientist	LIG, NIAID
OTHER:	P.A. Roche, Ph.D.	Staff Fellow	LIG, NIAID
	M. Malnati, M.D.	Visiting Associate	LIG, NIAID
	V. Pinet, Ph.D.	Visiting Fellow	LIG, NIAID
	M. Gueguen, Ph.D.	Visiting Fellow	LIG, NIAID
	P. Warmerdam, Ph.D.	Visiting Fellow	LIG, NIAID
	S. Rojo, Ph.D.	Visiting Fellow	LIG, NIAID

COOPERATING UNITS *(if any)*

W. Biddison, Neuroimmunology Branch, NINDS, NIH; R. DeMars, University of Wisconsin, Madison Wisconsin, O. Bakke, University of Oslo, Norway

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	4.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most immune responses occur only if a T lymphocyte recognizes foreign antigen in association with a self class II molecule of the major histocompatibility complex (MHC). Antigen must be processed for presentation by class II molecules and this processing generally involves endocytosis of antigen into an acidic compartment where newly synthesized class II molecules have been transported. The foreign peptide/MHC class II complexes recognized by CD4 T cells are thus formed intracellularly. The aim of this project is to define the potential sources of antigen for presentation by class II molecules, and to determine how class II molecules are transported to antigen-processing compartments. A genetic approach was used to demonstrate that several processing pathways exist for antigen presentation by class II molecules. The classical pathway for presentation of exogenous antigen requires newly synthesized class II molecules and a function encoded by an unknown gene within the class II region of the MHC. However, presentation of the influenza virus hemagglutinin antigen was independent of protein synthesis and of the function encoded by the unknown gene, implying that two processing pathways exist. In addition, it was demonstrated that two endogenous processing pathways lead to the presentation of cytosolic antigen to class II-restricted T cells, one is dependent on the transporter for antigen presentation (TAP), the other is not. In addition, the TAP-independent pathway requires another function that maps within the class II region of the MHC. This previously unsuspected presentation of cytosolic proteins by class II molecules has important implications for T cell repertoire selection, T cell tolerance, autoimmunity and vaccine design. Targeting of newly synthesized class II molecules to endosomes is mediated by the invariant chain (Ii), but the intracellular transport route is not known. This project demonstrated that a large population of class II-Ii complexes reach endosomes by rapid internalization from the cell surface. The highly efficient endocytosis was mediated by the cytoplasmic tail of Ii. Following internalization, Ii dissociated from class II-Ii complexes, suggesting that this pathway represent an important mechanism for loading class II molecules with peptides from several endocytic compartments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00171-16

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Infection of Rabbits with Human Immunodeficiency Virus 1

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. J. Kindt	Chief	LIG, NIAID
Other:	S. Cho	Visiting Fellow	LIG, NIAID
	B.F. Hague	Staff Fellow	LIG, NIAID
	R.M. Simpson	Expert	LIG, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS: 5	PROFESSIONAL: 3	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infection in rabbits injected with HIV-1 has been detected by seroconversion, detection of virus in cells and organs using PCR and *in situ* hybridization, and by isolation of virus from PBMC. While the fact that rabbits can be infected with HIV is promising, the slow course of infection, difficulty in isolation of virus and the absence of consistent disease signs in infected rabbits have limited utility of this model for AIDS. Information gained in recent efforts aim to improve the model suggest that infection in the rabbit may be enhanced in several ways. Adaptation of HIV to grow more efficiently in rabbits has been attempted by long term *in vivo* passage; injection of as little as 1 ml of blood from animals receiving splenocytes from a rabbit infected with HIV-1 and held for 2.3 years has resulted in lymphopenia, CD4 T cell depletion and for 2 of 12 rabbits, death. Initial results from structural analyses of HIV-1 amplified from a spleen cell recipient shows nucleotide sequence identity of 60-85% to the original input isolate, HIV_{Lai}. Compared to HIV-1, HTLV-I infects rabbit cells with high efficiency. Comparison of Northern Blots from HIV-1 and HTLV-I infected rabbit cells indicated that HIV-1 transcripts are relatively unstable, while those of HTLV-1 are as stable as endogenous message. The efficacy fo HTLV-1 infection in rabbit cells has prompted preparation of CAT constructs utilizing the 5' or 3' LTRS from HIV-1 or HTLV-I to provide the promoter or 3' processing signals. These are being used to compare LTR-mediated gene expression in rabbit and human cells. Data from these expression studies will be used to guide the construction of HIV-1 clones containing alternative LTR sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00180-15

PERIOD COVERED

October 1, 1992 TO September 30, 1993

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Properties of Transformed Rabbit Cell Lines

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.J. Kindt	Chief	LIG, NIAID
Other:	T.M. Zhao	Staff Fellow	LIG, NIAID
	R.M. Simpson	Expert	LIG, NIAID
	M. Leno	Visiting Fellow	LIG, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

A series of HTLV-I cell lines were prepared by in vitro transformation of rabbit peripheral blood mononuclear cells (PBMC) using HTLV-I cell lines of human or rabbit origin as virus source. It was found that certain infected lines killed rabbits in a dose dependent manner; animals injected with the lethal lines developed high temperature within four days of injection and usually died by day 8. A detailed description of organs from the affected animals is in progress; it appears that the HTLV-I lines cause infiltration of lung, lymph nodes, spleen and liver in a manner similar to adult T cell leukemia lymphoma in humans. Injection of irradiated cells was not lethal within the same short time frame as live cells, but most hallmarks of the disease were present. The majority of rabbit HTLV-I cell lines including several derived in the same fashion as the lethal line cause no overt disease. A detailed study of this phenomenon is underway; results here can shed light on why HTLV-I infection in humans causes serious disease in only a small percentage of infected individuals whereas most infected persons remain asymptomatic. Progress has been made in comparing lethal and nonlethal cell lines including a) structure of virus in lethal and non lethal cell lines; b) Characterization of the lines as CD4-, CD8-, $\gamma\delta$ TCR T cells c) disease caused by lethal lines has been shgown to be similar to human adult T cell leukemia/ lymphoma; d) host responses to lethal vs. nonlethal lines show difference in immune reactivity at the cellular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00389-09

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genotype Analyses of HLA and TCR Genes in Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Robinson	Senior Investigator	LIG, NIAID
Other:	K. Barron	IPA	LIG, NIAID
	H. Deulofeut	Visiting Scientist	LIG, NIAID
	C. Day	Senior Staff Fellow	LIG, NIAID

COOPERATING UNITS *(if any)*

D. Bernard Amos, Duke University, N.C.; P. Concannon, Virginia Mason Institute, Seattle, WA; P. Lusso, NCI

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.4	2.8	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inheritance patterns of HLA and T cell receptor (TCR alpha and TCR beta) genes which are unknown to play important roles in a variety of immune processes have been analyzed in human families. The extent of the TCR repertoire was investigated by analysis of cDNA and genomic libraries prepared from DNA samples derived from the members of families well characterized for HLA. The present studies have revealed both extensions and limitations of TCR V alpha and V beta repertoires. The known repertoire has been extended bay identification of new V alpha and V beta families, new members of defined families and allelic polymorphism in V genes segments. On the other hand, certain V genes have been characterized as pseudogenes which limits TCR repertoires. Six V beta genes were found to be encoded on chromosome 9 outside of the TCR beta gene complex which is encoded on chromosome 7. These genes, designated orphons, have sequences 93-97% identical to V beta genes within the genes complex, show no obvious defects that would preclude expression, but are not detected as rearranged transcripts with C beta. Two frequently occurring insertion/deletion related polymorphisms (IDRP) were found in the TCR beta gene complex; one involves a stretch of ~30kb in the V region and another spans ~20kb near the C region. Three TCR V beta genes have been localized to the V region IDRP; one gene is a previously uncharacterized member of a V beta family, the second gene is a precise duplication of a v beta gene also present in deleted TCR beta haplotypes and the third gene is a pseudogene. Three different V beta genes were found to have unexpressed or null alleles. Holes in the TCR repertoire caused by these unexpressed TCR alleles appear to contribute to susceptibility to a number of clinical subsets of JRA. Allelic variation in genes encoded within the HLA Class II region may contribute to susceptibility to Reiter's Syndrome. TAP genes are thought to function as transporters of peptides for presentation by Class I molecules and may contribute to susceptibility to disease by influencing the selection of peptides. The frequency of the TAP2A allele was increased in the patient group as compared to control groups and all patients were TAP2A+. Certain HLA-DQ alleles were over-represented in the patient group as well. Detailed knowledge of the extent and diversity of the germline TCR repertoire will greatly facilitate our ability to understand the role of TCR genes in immune responses in normal and disease states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00525-06

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Human Natural Killer Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.O. Long, Ph.D.	Visiting Scientist	LIG, NIAID
Other:	M. Malnati, M.D.	Visiting Associate	LIG, NIAID
	N. Wagtmann, Ph.D.	Visiting Fellow	LIG, NIAID

COOPERATING UNITS *(if any)*

P. Lusso, Lab. Tumor Cell Biology, NCI, NIH; and L. Moretta, National Institute for Cancer Research Genova, Italy

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Natural Killer (NK) cells are thought to play a role in the control of viral infection before the establishment of a specific cytolytic T cell response mediated by CD3-positive MHC-restricted T cells. Recently, specific recognition of alloantigens by NK cells was reported. Most of the cells displaying NK activity belong to the $CD3^-CD16^+CD56^+$ subset of peripheral blood lymphocytes. The mechanism of target cell recognition by NK cells is still unknown. The aim of this study was (i) to determine whether target cell elements control the recognition and lysis by NK cells, and (ii) to purify a surface molecule from NK cells in sufficient amount to obtain amino-acid sequences. As a first step, it was established that NK clones from a normal individual were able to specifically recognize and kill normal cells from the same individual that had been infected in vitro with human Herpesvirus-6 (HHV-6). The results showed for the first time that specificity in target cell recognition by NK cells is controlled at several levels: first, at the level of the NK clone itself, and second, by genetically variable elements on the target cells that restrict recognition by NK cells. By use of a monoclonal antibody, a NK cell surface protein that is involved in target cell recognition was purified and partial amino-acid sequences were obtained. The ultimate goal of this project is to obtain molecular clones for this molecule and to define its role in target cell recognition.

PHS 6040 (Rev. 5/92)

LABORATORY OF IMMUNOPATHOLOGY

1993 Annual Report
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Summary Statement
LABORATORY OF IMMUNOPATHOLOGY
National Institute of Allergy and Infectious Diseases
October 1, 1992 to September 30, 1993

The programs of this Laboratory have long been centered on various aspects of viral pathogenesis with increasing emphasis of late on the interplay between the immune system and 1) murine leukemia viruses (MuLV) and 2) cells transformed by papovaviruses, particularly adenoviruses. Also continuing are studies of hematopoietic differentiation with particular weight being given to studies of B cell development. The major accomplishments of the Laboratory in pursuit of these studies are as follows:

Retrovirus-induced immunodeficiency. Mice infected with a unique set of retroviruses, termed LP-BM5 MuLV, develop a syndrome of lymphoproliferation and increasingly severe immunodeficiency termed MAIDS. Studies of MAIDS have continued to dominate the activities of the Virology and Cellular Immunology and Viral Oncology Sections. In the last year, it has become increasingly clear that the etiologic defective virus (BM5def) that encodes a variant gag polyprotein must be present at levels above some threshold for MAIDS to develop with rapidity. At best, only low levels of expression are achieved in mice resistant to MAIDS due to MHC-linked genes (Hartley, Makino, Morse, Chattopadhyay) or in mice that bear any of as series of naturally occurring or induced mutations. Thus, mice expressing the *xid* mutation develop MAIDS with prolonged latency and have limited expression of BM5def (Tang, Hügin, Hartley, Morse). This effect may be due to reduced numbers of B cells as targets for infection by BM5def as suggested by the finding that mice with no B cells as the result of a targeted mutation of the IgM gene also have little or any BM5def in spleen (Kim, Morse). It is also possible that viral interference could inhibit induction of MAIDS in *xid* mice since endogenous ecotropic viruses are activated in mutant mice on the B6, C3H and BALB/c backgrounds (Tang, Torrey, Morse, Hartley). In addition, it would appear crucial not only that B cells are present in sufficient number but that they express MHC class II molecules since class II deficient mice do not develop MAIDS (Giese, Morse). The role of MHC class I genes is apparently to induce efficient CTL responses to eliminate BM5def infected cells (Makino, Morse, Hartley) but other responses can be equally protective since at least one strain of class I deficient animals is markedly resistant to induction of MAIDS (Tang, Hartley, Morse).

The mechanisms by which MHC class I or class II genes contribute to the susceptible/resistant phenotypes of different strains are not known, but one facet may relate to the patterns of $CD4^+$ T cell cytokine expression induced by expression of BM5def antigens in association with different alleles at class II loci. For example, infection of susceptible B6 mice is associated with production of Th2 cytokines (IL-4, -10) whereas resistant BALB/c mice express Th1 cytokines (IL-2, IFN-γ, (Giese, Gazzinelli, Morse). These different patterns of T cell activation and cytokine production provide an explanation for the decreased resistance of MAIDS-susceptible mice to infectious agents, such as *T. Gondii*, that normally are controlled by IFN-γ production. Resistance to these agents exhibited by mice with early MAIDS probably reflects induction by BM5def of IL-12, which in turn is a very efficient inducer of IFN-γ (Giese, Gazzinelli, Morse).

Efforts to better understand the effects of the gag product of BM5def have included the generation of mice bearing the BM5def gag gene as a transgene. Initial efforts yielded transgene-positive founders that failed to express protein. A second round of transgenics has just been produced (Amory, Morse Chattopadhyay). The same construct used for the transgenics has also been used to transfect lymphoma lines yielding expression of the gag protein (Amory, Morse, Chattopadhyay). Finally, gag constructs driven by CMV promoters have been used in gene gun experiments to determine if mice can be immunized against the gag protein to induce resistance to MAIDS (Amory, Kim).

In studies unrelated to MAIDS but involving retroviral pathogenesis, it has been found that MuLV host range variants can develop rapidly both in vivo and in vitro and that these variants may be have differing pathogenicities when used to infect mice (Torrey, Hartley).

DNA tumor viruses. This year has seen the distillation of long term efforts by members of the Viral Pathogenesis section and its collaborators to develop a biometric system for quantifying the tumorigenicity of transformed cell (Lewis, Alling, Banks, Cook). A series of papers were submitted that review the characteristics of tumorigenicity revealed in studies of DNA virus transformed cells transplanted in hosts of varying immune competence. Evaluations of tumor incidence and time of tumor appearance in animals given graded doses of cells have made it possible to define the tumorigenic phenotype of a specific line with great precision and to relate its phenotype to a variety of other lines examined in the same system. The paradigm holds great promise for associating particular tumor traits with the effects of specific viral or cellular oncogenes or anti-oncogenes (Lewis et al.).

Involvement of the p53 tumor suppressor gene has been implicated in adenovirus (Ad) tumor progression by recent studies of a series of transformed cells. Studies of one Ad5 transformed line revealed a mutation at codon 156 of p53, an unprecedented occurrence in mouse tumors (Eyler, Lewis, Huppi).

Hematopoietic differentiation. Using a series of monoclonal antibodies developed in this laboratory and elsewhere, it has been possible to associate changes in cell surface antigen expression with states of differentiation as indicated by gene organization and histopathology. LIP-6 is an antibody that defines a disulfide linked complex of 75, 65 and 43 kDa on the surface of B lineage cells and macrophages. In the bone marrow, this antigen is expressed on cells with the ability to differentiate into B cells or myeloid cells. Studies of splenic B cells showed that crosslinking of this molecule stimulated B cell proliferation (Holmes). In the later stages of B cell differentiation, the antigen is expressed on immunoblasts and early plasma cells but not mature plasma cells which are recognized by anti-CD43. LIP-6 antigen is being prepared in quantity to permit microsequencing and the identification of the gene (Lantz, Holmes)

Administrative changes. Dr. Sisir Chattopadhyay was awarded tenure in NIAID as a Staff Scientist. Dr. Marjorie Shapiro left the Laboratory to join the FDA in CBER. Dr. Ted Torrey joined the Viral Oncology Section as a Senior Staff Fellow and Dr. Renate Morawetz came to the Virology and Cellular Immunology Section as a Fogarty Visiting Fellow.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00013-30 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology and Pathogenesis of DNA Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Andrew M. Lewis Jr, M.D., LIP
Others: Yvonne Eyler, LIP
David Alling, ODIR

COOPERATING UNITS *(if any)*

National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado (J.L. Cook); New York State Office of Mental Health (S. Banks).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 2.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

After 5 years of intense effort, we have developed a new approach to study the contribution of viral oncogenes to transformed cell tumorigenicity. This approach defines those viral oncogene induced traits expressed by transformed cells that determine their tumorigenicity in terms of mean tumor producing dose 50% end point (TPD50) values which are estimates of the number of cells required for tumor formation. By determining these values at weekly intervals, in different types of animals that possess different capacities to mount antitumor defenses, the tumorigenic phenotype expressed by transformed cells can be characterized in terms of the kinetics of the tumor cell dose-tumor incidence-tumor latency relationship. Analyzing and comparing these relationships for cells transformed by different viral oncogenes provides methods for: 1) discriminating between oncogene-induced traits that determine tumorigenicity and random low frequency events; 2) detecting tumor latency traits that differ from those traits that determine tumor inducing capacity; 3) demonstrating that viral oncogenes contribute traits in addition to immortalization that are responsible for a transformed cell's tumor inducing capacity; 4) organizing oncogene-induced traits necessary for tumor formation into host responsive and host unresponsive categories; and 5) ranking DNA virus transformed cells according to their relative aggressiveness as tumor cells from nontumorigenic to highly tumorigenic cells that metastasize. This approach integrates in vitro and in vivo models of neoplasia and provides a means of standardizing the definition of tumorigenicity and the measurement of neoplastic processes in experimental models whose endpoints can be analyzed statistically. The application of this approach will contribute to our understanding neoplastic processes and in managing their consequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00135-16 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Properties of Immunoglobulin Secreting Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, M.D., Chief, LIP, NIAID
Others: U.R. Rapp, Senior Investigator, LVC, NCI
J.H. Pierce, Senior Investigator, LCMB, NCI
K.L. Holmes, Senior Investigator, BRB, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00138-16 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Viruses and Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, M.D., Chief, LIP, NIAID
Others: Janet Hartley, LIP
Brian Murphy, LID
Arun Kulkarni, LID
Mark Connors, LID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The central goal of this project is to understand the mechanisms by which the immune system responds to different viral infections by generating either a protective or curative response or an immunopathologic response. Most of the work this last year was performed in LID with the collaboration of Dr. Brian Murphy and his group on studies of respiratory syncytial virus. The results are summarized in project number ZO1 AI 00345-12 LID and will not be repeated here.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00205-09 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Abnormalities of T and B Lymphocytes of Autoimmune Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, M.D., Chief, LIP

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00284-12 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Pathogenic Murine Leukemia Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Janet W. Hartley, Head, Viral Oncology Section, LIP
Others: Herbert C. Morse III, LIP
Torgny Fredrickson, NCI
Sisir Chattopadhyay, LIP
Yao Tang, LIP
Ted Torrey, LIP

COOPERATING UNITS *(if any)*

National Institute of Health in Japan (M. Makino); C. Hausen, VRP, RR, J. Kenny, NCI-FCRDC, FDA (A. Rosenberg, A. Khan), Laboratory of Neuroscience, NIDDK (Y. Sei)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project deals with the biological and molecular characterization of murine leukemia viruses (MuLV) with the goal of understanding host and viral factors involved in the pathogenesis of neoplastic and non-neoplastic sequelae of infection. Primary emphasis is on the complex of viruses (LP-BM5 MuLV) responsible for induction of murine AIDS (MAIDS). Infection of sensitive strains of mice with this mixed virus stock, which contains a 4.9kb replication defective genome (BM5def) and B-tropic replication competent ecotropic and MCF helper MuLVs, results in progressive lymphoproliferative disease and immune system impairment and, mostly in mice held long-term, both B and T cell lymphomas can be detected by transfer to Scid mice or, occasionally in situ, by FACS analysis or histopathology. The disease process requires BM5def and both B cells and CD4^{+} T cells. A BM5def genome containing a viral construct which permits the defective genome and a gene for neomycin resistance to be concordantly expressed has been used to transfect the packaging cell line GP+E-86 to provide a stock of defective, helper-free virus. Although C57BL mice are highly sensitive to development of MAIDS, B6 congenic for the X-linked immunodeficiency (xid) gene are resistant. Uninfected B6.xid mice were found to express high levels of ecotropic MuLV as early as 8 wk of age, much earlier than normal B6. The isolates were B-tropic, in contrast to the usual N-tropism of induced B6 endogenous MuLV sequences. These findings have been extended to BALB.xid, although the frequency of virus expression is much lower, and early expression of virus has been found in Fv-I^{n} C3H.xid. The 2 mechanism of early virus expression and its B-tropism in Fv-1^{b} strains is under study as well as determination of whether the basis for B6.xid resistance to MAIDS is virologic or immunologic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00286-12 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Janet W. Hartley, Head, Viral Oncology Section, LIP, NIAID
Others: Herbert C. Morse, LIP
Torgny Fredrickson, NCI
Yao Tang, LIP
Ted Torrey, LIP
Sisir Chattopadhyay, LIP

COOPERATING UNITS *(if any)*

National Institute of Health in Japan, NIAID (R. Schwartz), NY State Dept. of Health, Jackson Laboratory, Whitehead Institute, Northwestern University Medical School.

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.9	PROFESSIONAL: 1.8	OTHER: 1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study host genetic determinants of infection of inbred mice by murine leukemia viruses (MuLVs), emphasizing the genetically controlled elements affecting induction of MAIDS by replication defective BM5def MuLV. In the case of MAIDS, strain differences in susceptibility reflect the function of host genes that lie both within and outside the MHC complex and control spread of helper virus and/or the activity of the BM5def genome itself. As determined by infection of Fv-1^b mouse strains, whose cells are permissive for the B-tropic helper viruses in LP-BM5 MuLV, the H-2 haplotypes b, p and s were among the most sensitive to MAIDS while haplotypes a and d were highly resistant. In H-2^d mice, resistance was mapped to the D end of H-2 but this association was inconsistently detected, depending on the background strain. Expression of the MHC Class I I-region Ea gene product has now been shown to modify Class II effects, based on patterns of sensitivity in H-2 recombinant strains and the finding that C57BL/6 transgenic for Ead are much more resistant to MAIDS than the parental mice. Class I-modified resistance does not appear to reflect the suppression of helper virus seen in some resistant strains. In Fv-1^n strains, studied by infection with BM5def pseudotyped by N-tropic amphotropic MuLV, development of MAIDS is closely linked to H-2 (haplotypes b and p are sensitive) and to permissiveness of adults to amphotropic virus. In a few strains, however, non-MHC effects can be seen in several instances of delayed disease induction. Spontaneous lymphoid tumors in NFS mice congenic for ecotropic virus induction loci have been used in studies of the correlation of diagnostic outcomes of analysis by histopathology, FACS profiles, immunocytochemistry and molecular probing for Ig and TCR rearrangements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00465-08 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Retrovirus-Induced Murine Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, Chief, LIP
Others: Janet W. Hartley, LIP
Sisir Chattopadhyay, LIP
Yao Tang, LIP
Nathalia Giese, LIP
Wan Kee Kim, LIP
Torgny Fredrickson, NCI
Renate Morawetz, LIP
R. Gazzinelli, LPD
F.A. Sher, LPD
L. Selvey, EIB, NCI
R. Hodes, EIB, NCI

COOPERATING UNITS *(if any)*

Immuno AG; Univ. Hospital; Geneva; Natl. Inst. of Health, Japan; DNAX; Genetics Inst; Univ. of Cologne; Hoffman La Roche, Genentech.

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to develop a basic understanding of the pathogenesis of retrovirus-induced immunodeficiency with successful prevention and treatment of disease being the ultimate goals. The LP-BM5 mixture of murine leukemia viruses (MuLV) induces an immunodeficiency syndrome termed mouse AIDS or MAIDS in mice of susceptible strains. Previous in vitro studies suggested that the disease-causing defective virus in this mixture exerts a superantigen-like effect. In vivo evidence for a superantigen effect was provided by the observation of preferential activation and expansion of $V\beta5^+$ $CD4^+$ T cells during the early stages of MAIDS. Genes of the major histocompatibility complex are major determinants of susceptibility or resistance to MAIDS. In resistant strains, their effects are mediated by induction of protective $CD8^+$ T cell dependent immunity in resistant mice in association with a Th1 pattern of cytokine expression (IL-2, IFN-γ) by $CD4^+$ cells. In susceptible mice, a Th2 pattern of cytokines was found to predominate (IL-4, IL-10). These polarized responses to infection may be driven by interaction of the defective virus antigen with various MHC haplotypes and nonspecific responses of the immune system including production of IL-12 and TNF-α by macrophages. Studies are in progress to manipulate the immune system of MAIDS-susceptible mice towards a Th1 response to induce resistance to disease. Additional studies are directed at defining other parameters of disease susceptibility using a series of gene knockout and transgenic mice. Preliminary studies demonstrate that disease does not develop in mice lacking MHC class II molecules or in mice lacking B cells, strongly supporting the suggestion that the disease is driven by association of viral antigen/MHC class II complexes specifically on B cells.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00484-07 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms in Hematopoietic Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Kevin L. Holmes, BRB, NIAID
Others: H.C. Morse III, LIP
L. Staudt, DCBD, NCI
P. Sherle, DCBD, NCI
J. Taubenberger, LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	2.0	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall goal of this project is to promote a clearer understanding of the mechanisms of hematopoietic cell differentiation by characterizing cell surface antigens expressed during the development, and after the maturation, of the blood elements. We have primarily focused on the characterization of a novel antigen identified by a monoclonal antibody LIP-6. LIP-6 was produced against an undifferentiated murine hematopoietic cell line in order to identify antigens expressed early in development. The LIP-6 antigen is composed of three disulphide-linked proteins that are expressed by bone marrow cells, B lineage cells and macrophages but absent on all T lineage cells. Antibodies to LIP-6 can induce proliferation of mature B cells in the absence of T cells, suggesting that LIP-6 may be important in activation of mature B cells. A population of bone marrow cells, identified by its expression of LIP-6, has been found to be an immature progenitor population with limited differentiation capacity within the B, T and myeloid cell lineages. Future studies will attempt to isolate the gene coding for the LIP-6 protein and further characterize the population of cells expressing LIP-6 in bone marrow. These studies will hopefully yield new information about the cellular interactions important in B development and B lymphocyte interactions. Two other cell surface antigens expressed on bone marrow, BRB44 and Ly-26 are also being studied. BRB44, produced in our laboratory against an undifferentiated cell line, has a very complex strain distribution pattern among inbred strains of mice and is expressed on mature B and myeloid lineage cells. The BRB44 antibody precipitates a 72kDa, glycosylated protein. Recent characterization of Ly-26.1, has shown that it is variably expressed on most bone marrow cells, mature B lymphocytes and granulocytes/macrophages and on CD8 T cells. Anti-Ly-26 antibodies precipitate 100 or 120 kDa proteins, dependent upon tissue localization.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AI 00544-05 LIP

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Basis of Pathogenesis of Murine Leukemia Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Sisir K. Chattopadhyay, LIP, NIAID
Others: H.C. Morse III, LIP
J.W. Hartley, LIP
Torgny Fredrickson, NCI
Yao Tang, LIP
Wan Kee Kim, LIP
John Amory, LIP

COOPERATING UNITS *(if any)*
Veterans Admin., Baltimore, MD (J. Eiseman, J. Bilello), Yale Univ. (R. Flavell), Albert Einstein College of Med. (J. Lenz)

LAB/BRANCH
Laboratory of Immunopathology

SECTION
Virology and Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have shown that a 4.9kb replication defective recombinant murine leukemia virus (MuLV), present in the LP-BM5 murine leukemia virus complex, is the proximal cause of a lymphoproliferative, immunodeficiency syndrome, murine AIDS (MAIDS). The defective virus (BM5def) was molecularly cloned and sequenced to reveal that the virus has a functional LTR and the gag gene is intact. Within the gag gene only the p15 and p12 are highly divergent from other MuLV. In studies of the role of gag protein(s) in pathogenicity we have found that although bacterially expressed p12 gag induced high levels of antibodies in both susceptible and resistant mice there was no induction of a protective immune response. In order to determine the consequences of constitutive expression we prepared 2 constructs of BM5 def gag for use in establishing transgenic mice. One utilized SV40 promoter and immunoglobulin (Ig) heavy chain enhancer to direct the gag gene expression in B cells and although 1/8 founder mice expressed the transgene, it did not transmit through the germline. The second construct utilizes MHC class II (Ea) promoter, to allow expression in B cells, macrophages and dendritic cells. Selection of transgene positive mice from the Ea promoter construct is in progress. We also developed two gag gene expression vectors (one with β actin promoter and the other with CMV promoter) and used them to transfect B cell lineage tumor cell lines. None of the surface-positive transfectants were able to stimulate the proliferation of normal spleen cells. We are continuing such studies with other promoters. To explore the effects on pathogenicity of alterations in the structure of various segments of the BM5def genome, we have constructed a biologically active genomes containing LTR enhancer elements from a highly leukemogenic ecotropic virus and are preparing chimeric genome containing non-pathogenic ecotropic elements. We used a 100bp probe derived from the p12 gag region of BM5def to demonstrate its presence in the tissues of infected mice by restriction endonuclease digestion and southern blotting. In some instances, especially when a defective virus containing a bacterial neomycin resistance gene was used to induce disease, we were unable to detect the defective viral genome in the involved tissues by similar techniques. We thus developed primers and probes to detect defective genomes by PCR reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00578-03 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Oncogenes in B Cell Neoplasia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Shapiro, LIP, NIAID

COOPERATING UNITS *(if any)*

Institute of Medical and Veterinary Science, Adelaide, South Australia (W. Langdon)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00692-01 LIP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Determinants of Murine Leukemia Virus Pathogenicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ted A. Torrey, LIP, NIAID
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INSTITUTE AND LOCATION

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project aims to elucidate the molecular nature of differences in murine leukemia virus (MuLV) infective host range in vitro, and pathogenicity in vivo, in the hope that characterization of the determinants responsible for the restriction of retroviral replication will prove generally enlightening. As a model system, we are examining an ecotropic MuLV (named RF.AMLV) which, along with the Moloney ecotropic MuLV (MoMuLV eco), is greatly restricted for in vitro infection of a Mus dunni mouse fibroblast cell line. This is quite unusual, since the Mus dunni cell line is highly sensitive to infection by most ecotropic MuLV and indeed to many xenotropic, amphotropic and mink cell focus-inducing (MCF) MuLV. Successive serial passage of the RF.AMLV through the dunni cell line gave rise to virus stocks which have a greatly altered in vitro tropism profile: in addition to having acquired the ability to infect the Mus dunni cell line, these stocks have lost the ability to infect certain other mouse cell lines, notably the Mus musculus domesticus wild mouse SC-1 cell line which is extraordinarily sensitive to MuLV infection. Strikingly, infection of neonatal mice with RF.AMLV or MoMuLV eco also results in the generation of similar ecotropic variants (i.e. acquired dunni-tropism) in vivo. In addition, mice of strains which express high levels of endogenous ecotropic MuLV demonstrate an age-dependent increase in the percentage of these ecotropic variants recoverable from various tissues. Studies indicate that 1) pseudotyping of the ecotropic MuLV mink cell focus-inducing (MCF) MuLV is not responsible for the observed change in infective host range; 2) no major recombination event detectable by restriction endonuclease mapping or differential hybridization is involved; and 3) significant differences in the latency and histopathology of the disease induced by various RF.AMLV stocks appears to correlate with the percentage of different ecotropic variants within the viral population.

Laboratory of Immunoregulation
1993 Annual Report
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Summary Report
Laboratory of Immunoregulation
October 1, 1992 through September 30, 1993

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation

Over the past several years LIR has been involved in the delineation of the immunopathogenic mechanisms of human immunodeficiency virus (HIV) infection and disease. We have reached the conclusion from our studies that the pathogenic mechanisms of HIV disease are multifactorial and multiphasic, and that these phases overlap throughout the prolonged course of HIV infection. It has become clear that only by appreciating in a comprehensive manner the complex immunopathogenic mechanisms of disease can we form a solid scientific basis for the design of therapeutic and vaccine strategies. In this regard, we have investigated the immunopathogenic mechanisms of HIV disease at the tissue, cellular, cytokine and molecular levels. In addition, we have designed and executed therapeutic and vaccine protocols.

The Role of Lymphoid Tissue in the Pathogenesis of HIV-1 Infection

Primary HIV-1 infection is generally associated with a burst of viremia with or without clinical symptoms, and is followed by a prolonged period of clinical latency. During this period viremia declines dramatically, the numbers of infected cells in the blood are very low, and it is extremely difficult to demonstrate virus expression in these cells. We have analyzed viral burden and levels of virus replication simultaneously in the blood and lymphoid organs of the same individuals at various stages of HIV disease. We have shown that in early stage disease there is a dichotomy between the levels of viral burden and virus replication in peripheral blood versus lymphoid organs. The histopathologic abnormalities [i.e. follicular hyperplasia and expansion of the follicular dendritic cells (FDC) network] are responsible for the trapping of virions within the FDC network and sequestration of HIV-infected cells in the lymphoid organs in early stage disease. As disease progresses there is a disruption of the tissue architecture and most of the germinal centers are involuted concomitant with a loss of the ability of the node to trap virus, which is caused by the dissolution of the FDC network. Therefore, in late stages of disease the mechanical mechanisms of virus trapping and sequestration of infected cells are removed and an effective immune response to HIV is lost; this leads to an equilibration of the viral burden and replication between peripheral blood and lymphoid organs. These results indicate that HIV disease is active in the lymphoid tissue throughout the period of clinical latency, even at times when minimal viral activity is demonstrated in the blood.

Gut associated lymphoid tissue represents the major reservoir (50-60%) of total lymphocytes in humans. Lymphoglandular complexes are disseminated throughout the gut. These lymphoglandular complexes are immunologically functional structures. Germinal center formation may occur in these lymphoglandular complexes and the presence of B cells, T cells, FDCs, and macrophages indicates that antigen presentation may occur in these structures. We have analyzed by *in situ* hybridization the presence and the distribution of HIV-1 RNA in the gut.

Preliminary analyses have demonstrated a distribution of HIV-1 RNA similar to that observed in lymph nodes. Most of the hybridization signal corresponded to extracellular viral particles, and was restricted to the FDC network, whereas isolated cells expressing HIV-1 RNA are only rarely found outside the germinal centers.

We have analyzed the expression of a group of cytokines including interleukin (IL)-2, IL-4, IL-6, IL-10, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) in unfractionated mononuclear cells and sorted $CD8^+$, $CD8^-$, $CD4^+$ and $CD4^-$ T lymphocytes isolated from peripheral blood and lymphoid tissue of the same HIV-1 infected individuals by quantitative polymerase chain reaction (PCR). Levels of IL-2 and IFN-γ expression are higher in lymph nodes compared to peripheral blood, and the levels of expression of these cytokines decrease with disease progression. Levels of expression of IL-10 and TNF-α are slightly higher in lymph nodes versus peripheral blood and do not seem to be influenced by the different stages of disease. Expression of IL-6 is significantly higher in lymph nodes versus peripheral blood in early stage disease. However, IL-6 expression progressively increased in peripheral blood with disease progression. Expression of IL-4 was not detected in both peripheral blood and lymph nodes in any stage of disease. Analysis of cytokine expression in sorted $CD8^+$, $CD8^-$, $CD4^+$ and $CD4^-$ T cell subsets has demonstrated that $CD8^+$ T cells are the cells responsible *in vivo* for the production of IFN-γ, and, at least in part, for the production of IL-10. These results indicate that there is a progressive decline of IL-2 and IFN-γ levels associated with disease progression. More importantly, $CD8^+$ T lymphocytes but not $CD4^+$ T cells, are the cells responsible for the production of IFN-γ *in vivo*, which is an important modulator of the immune response. These studies will lend insight into the role of cytokines in the regulation of HIV expression and in the progressive dysfunction of the immune system in HIV disease.

We have analyzed viral burden and expression in mononuclear cells isolated from the bronchoalveolar lavage (BAL) and peripheral blood from the same HIV-1 infected individuals by semiquantitative DNA and RNA PCR. Viral burden and replication were similar in mononuclear cells isolated from peripheral blood and BAL. A major difference between peripheral blood and BAL is represented by the different types of cells harboring HIV-1 in the two anatomic compartments. $CD4^+$ T cells are the major target of HIV-1 in the peripheral blood, whereas alveolar macrophages seem to be the major target of HIV-1 in the lung. (Pantaleo, Graziosi, O. Cohen, Muro-Cacho, Vaccarezza, Fauci, LIR, NIAID; Fox, Molecular Histology; Orenstein, GWU; Baseler, PRI, Frederick; Kotler, St. Luke's Hospital, NYC; Shaw, Saag, UAB, Birmingham).

The Role of the Thymus and Bone Marrow in the Immunopathogenesis of HIV Infection

We have utilized the SCID-hu mouse model to study HIV infection of the human thymus and have found that this tissue can be reproducibly infected with HIV after direct injection of the virus. A variety of thymocytes at different stages of maturation are infected including the CD4/CD8 double positive cells as well as the CD4 and CD8 single positive mature thymocytes. Depending on the viral isolate used, 10 to 80% of total thymocytes may become infected, and severe thymocyte depletion occurs. Additionally, the thymic epithelial (TE) cells, which is the major structural cell of the thymus and is vital for the normal maturation of thymocytes, also becomes infected in this model. Infection does not seem to result in TE cell death, so that these cells may be an important reservoir for maintaining infectious virus in this tissue. In addition to the direct infection of these cells by HIV, we observed an independent toxic effect on these cells resulting in degeneration and loss of this supporting stroma. We are now using the SCID mouse to develop models for the study of HIV infection in both lymph nodes and intestine, and have found that these tissues obtained from human fetuses can be transplanted into the SCID mouse and can be infected with HIV after intravenous injection of virus. Further studies will delineate the cell types which are infected. Preliminary studies to evaluate the role of the bone marrow as a reservoir for HIV have been inconclusive but do not suggest that the bone marrow functions in this capacity in most individuals. Evaluation of the integrity of the bone marrow stroma in infection is ongoing with initial results in one patient showing no significant disruption of the marrow stroma by HIV infection. (Stanley, Granberry, Fauci, LIR/NIAID; McCune, Kaneshima, SyStemix, Inc., Palo Alto; Fox, Molecular Histology, Rockville, MD; Orenstein, GWU; Baseler, PRI, Frederick, MD).

The Role of Dendritic Cells (DC) in the Immunopathogenesis of HIV Infection

We have found two populations of cells in peripheral blood that morphologically resemble DC. The first population is very potent in stimulating T-cells and is not infectable with HIV. The second population has low levels of Fcγ receptors and is productively infected with HIV *in vitro*. The finding of these two populations of DC-like cells may explain the contrary results reported in the literature concerning DC HIV infection *in vivo* and *in vitro* as well as DC dysfunction and depletion in advancing HIV disease. The purified FcγR negative DC when pulsed with HIV and added to unstimulated CD4$^+$ T-cells induce a vigorous infection of the T-cells. Electron microscopy of the HIV pulsed DC demonstrates viral particles bound to the surface of the cells. A model of the initiation of HIV infection where DC bind HIV in the periphery and carry the virus back to lymphoid tissue and infect T-cells is being developed. (Weissman, Fauci, LIR/NIAID; Fox, Molecular Histology; Orenstein, GWU; Tedder, Zhou, Dana-Farber, Boston, MA)

The Role of Cellular Activation in the Immunopathogenesis of HIV Infection

We have investigated the occurrence of apoptosis in peripheral blood mononuclear cells or in lymphoid tissues obtained from HIV infected individuals in different stages of disease. Two experimental approaches have been used: i) staining of cell suspensions with acridine orange for the detection of chromatin condensation, an early step in apoptosis, or formation of apoptotic bodies; ii) detection of fragmented DNA. Apoptosis has never been detected in freshly isolated peripheral blood mononuclear cells obtained from patients at any stage of disease. Preliminary results obtained in tissue sections of lymph nodes from HIV-1 infected individuals suggest that some cells may undergo apoptosis in tissues although these apoptotic cells seem to be immediately removed from the tissue. In this regard, apoptotic bodies may be detected in macrophages. These data indicate that the apoptosis phenomenon may occur *in vivo*.

By studying HIV-1 infections of normal T cells with primary clinical isolates, and by studying transfected T cell tumor lines, we established that pathogenic (syncytium-inducing=SI) HIV-1 induces a specific form of programmed cell death (PCD) characterized by cell cycle arrest at the G2/M cell cycle checkpoint. Antibodies to the G2/M cell cycle protein, cyclin B, identified individual cells dying of the cytopathic effect associated with SI HIV-1 infection. This process can now be studied in patients to attempt to learn what populations of T cells undergo this form of PCD, and to learn whether the percentage of affected cells can be used to predict progression to acquired immunodeficiency syndrome (AIDS). The aberrant HIV-initiated form of PCD contrasted substantially with normal PCD occurring during T cell development (negative selection) as demonstrated by differential sensitivity to metabolic inhibitors. The phosphatase 2A inhibitor, okadaic acid, and the protein tyrosine kinase inhibitor, herbimycin A were demonstrated to interfere with HIV-initiated cytopathicity. Overexpression of the Bcl-2 oncogene, which blocks the apoptotic form of PCD occurring during negative selection, could not inhibit HIV-induced PCD. These differences between HIV-initiated PCD and negative selection offer encouragement for the development of therapeutic agents capable of specifically inhibiting HIV-mediated PCD.

We have tested the effect of Cyclosporine A (CsA) on HIV-1 infection *in vitro*. Phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells cultured in the presence of IL-2 have been inoculated with HIV-1 MN strain or with primary isolates in the presence or absence of different concentrations of CsA. CsA at higher concentrations (10^{-6}M and 5 x 10^{-7}M) significantly (60-80%) inhibited virus replication as measured by reverse transcriptase (RT) activity, HIV spreading as indicated by DNA PCR, and exerted a protective effect on the depletion of $CD4^+$ T cells in culture. In a second series of experiments, suboptimal concentrations of CsA (generally 3 x 10^{-7}M) and AZT were used in combination. The results obtained indicated that suboptimal concentrations of CsA and zidovudine (AZT) used alone had only partial or minimal effect on HIV-1 infection, but used in combination completely suppressed HIV-1 infection *in vitro*. The concentrations of CsA exerting an inhibition on HIV-1 infection corresponded to easily tolerated serum levels of CsA used in the treatment of other diseases.

Since the expressed T-cell receptor repertoire is profoundly influenced by the human lymphotropic antigen complex and is not equally expressed in $CD4^+$ and $CD8^+$ T cell subsets, we have analyzed the Vβ repertoire in sorted $CD4^+$ T cell subsets obtained from 5 couples of monozygotic twins who were discordant for HIV-1, one of whom was HIV-positive and the other of whom HIV-negative. Comparative analysis of the Vβ repertoire in sorted $CD4^+$ T cells in each pair of twins showed that Vβ 2 and Vβ 3 were significantly decreased (50%) in the HIV-positive twin compared to the HIV-negative twin in three of five of the couples of twins analyzed. These results are consistent with the effects of a superantigen. However, they suggest that rather than cause deletions of specific subsets of T cells, it is more likely that superantigens, if present in HIV infection, serve as potent activators of T cells, rendering them more susceptible to infection with virus.

We have investigated whether ICAM-1, ICAM-2 and ICAM-3 molecules may function as counter-receptors for LFA-1 in the process of HIV-mediated syncytia formation. The involvement of the ICAM (1-2-3) molecules has been analyzed by blocking LFA-1/ICAMs (1-2-3) interaction with anti-ICAM-1/-2/-3 monoclonal antibodies (mAbs) used alone or in combination. Complete inhibition of syncytia formation was achieved only in the presence of the three anti-ICAMs (1-2-3). These results indicate that ICAM (1-2-3) molecules are the counter-receptors for LFA-1 in the process of HIV-mediated syncytia formation. We have also investigated the role of cell activation in the process of HIV-mediated syncytia formation. Syncytia formation occurred only when already HIV-infected PHA activated mononuclear cells were cocultured with PHA activated uninfected $CD4^+$ target cells. In contrast, when resting $CD4^+$ T cells were cocultured with infected PHA activated mononuclear cells syncytia formation was never detected. These results indicate that cell activation is required for HIV-mediated syncytia formation in primary $CD4^+$ T cells. (Pantaleo, Graziosi, Muro-Cacho, O. Cohen, Vaccarezza, D. Cohen, Fauci, LIR/NIAID; Fox, Molecular Histology, Rockville; Orenstein, GWU), Baseler, PRI, Frederick; Springer, deFourerolles, Harvard, Boston; Soudeyns, Rebai, Sekaly, Univ. of Montreal).

The Role of Endogenous and Exogenous Cytokines in the Regulation of HIV Expression

HIV infection *in vivo* is a systemic infection of T lymphocytes, and certain antigen presenting cells, particularly those of the monocyte/macrophage lineage. The characteristics of cytopathicity, expression or latency in these different cell types are very likely regulated by an interconnected network of virally encoded genes and host factors, including endogenous cytokines which can regulate both the immune and the inflammatory responses. Over the previous several years, the LIR has made the original observation that cytokines (both exogenous and endogenous) can modulate the expression of HIV *in vitro*. We have delineated the cellular and molecular mechanisms of these effects and have demonstrated the fact that endogenous cytokines can regulate HIV expression in an autocrine and paracrine manner. Our work demonstrates that both pro-inflammatory and immunoregulatory cytokines exert important effects on HIV expression in chronically infected cells, and replication in acutely infected primary cells. The role of NF-κB in HIV replication has been highlighted by studies in isogenic clones of U937 cells which revealed high or low efficiency of supporting HIV replication in correlation with the presence of either wild type or a shorter form of the p65 subunit of NF-κB. Furthermore, transdominant mutants of NF-κB have been identified which could be of potential use for gene therapy purposes. Pharmacologic agents which either mimic or functionally interfere with cytokine-dependent regulation of HIV expression [n-acetyl-cysteine (NAC), RP55778, pentoxifylline, IL-1ra] have been characterized *in vitro* and are of potential clinical interest. Studies in infected macaques reveal bursts of cytokines during the first weeks of infection in coincidence with the surge of plasma viremia, suggesting the possibility of interfering with viral spreading *in vivo* by cytokine or anti-cytokines strategies. Delineation of the precise scope and mechanisms of the regulation of HIV expression by cytokines will have important implications in the design of therapeutic strategies aimed at blocking endogenous cytokines which upregulate HIV expression or administering exogenous cytokines which down-regulate HIV expression. (Poli, Biswas, Weissman, L. Fox, Brown, Franzoso, Siebenlist, Bressler, Fauci, LIR/NIAID; Orenstein, GWU; Rabson, UMDNJ, Piscataway, NJ: C. Fox, Molecular Histology, Rockville, MD)

Regulation of HIV Gene Expression

Recently we have proposed a model for the mechanism of action of the *Rev* protein of HIV-1 which holds that *Rev* cooperatively binds with cellular factors and the *Rev*-Response Element (RRE) to form a functional complex. Using cooperative binding as a criteria to search for *Rev* accessory factors we have detected in crude extracts a nuclear activity which promotes the binding of *Rev* to the RRE. This activity either cooperatively binds to the RRE with *Rev* or catalyzes the binding of *Rev* to the RRE. Almost certainly the activity is protein. Chromatographic analysis of the extracts has resolved at least four and possibly more separate activities. One of the activities is now about 20% pure. The physico-chemical data we have accumulated concerning the others will enable us to rapidly purify them. While we are cloning them, we will be studying their functions by microinjecting them (as semi-pure fractions) into mammalian cells along with appropriate indicator genes. In related work we have studied the interaction of the *Rev* protein with the RRE. We have found that RRE determinants strongly influence the binding of Rev molecules subsequent to the first binding event. This precludes models for *Rev*/RRE binding which hold that all of the specificity is in the first binding event and that subsequent multimerization of *Rev* along an RNA molecule involves only non-specific recognition of RNA by *Rev* protein. In a new series of investigations we have isolated a series of cellular genes regulated by HIV infection in macrophages. Surprisingly the mRNA coding for the S16 ribosomal protein is downregulated. It will be interesting to see how this influences HIV expression. The upregulated genes include the endogenous human retrovirus-like element, Line-1, suggesting a possible evolutionary link between this endogenous virus and HIV. All upregulated genes will be evaluated for their suitability as therapeutic, immunologic targets. (E. Dayton, Powell, Hsu, Bende, Butini, Pantaleo, Zhang, A. Dayton, LIR/NIAID; Maizel, Shapiro, LMB/NCI; Gallo, Klotman, LTCB/NCI; Konings, U. Colorado; Baroudy, Kotwal, Gamble Institute, Cincinnati, OH)

Anti-Retroviral Therapy of HIV Infection

An intensive effort was directed toward studying the preventive and therapeutic aspects of HIV infection and AIDS. A randomized trial comparing therapy with zidovudine (ZDV) versus IFN-α versus the combination in 180 patients with early HIV-1 infection completed accrual and is currently active. A dose-tolerance study of the combination of IFN-α and dideoxyinosine (ddI) completed accrual of 53 patients and is now gathering long-term follow-up data. A randomized, double-blinded, placebo-controlled Phase II study of L-697,661 versus ZDV completed accrual of 84 patients using surrogate markers as efficacy parameters and is now in press. A randomized, dose escalation study of U-90152S in combination with ZDV and ddI versus ZDV and ddI alone has enrolled 18 patients and is enrolling at the third dose level. A dose-escalation study of PMEA has enrolled 12 patients and continues to enroll at the second dose level. A phase I study of CD4-Pseudomonas Exotoxin (sCD4-PE40) in HIV-infected patients was initiated and enrolled 24 patients to determine the pharmacokinetics, maximally tolerated dose, and preliminary efficacy of this agent. Follow-up analysis of a randomized, controlled, masked trial of foscarnet in the treatment of cytomegalovirus retinitis in AIDS patients demonstrated an apparent survival benefit of this therapy. 2-β-fluoro-dideoxyadenosine (FddA) was found to display markedly superior protection against infection relative to zidovudine in the SCID-hu-PBL model of HIV-1 infection. Whereas pre-treatment with ZDV still resulted in infection rates as high as 25%, pre-treatment with FddA prevented successful infection in all animals. FddA was also found to have utility in preventing successful infection when administered after infectious challenge. (Polis, Davey, Walker, Falloon, Siedel, Ruxrungtham, D. Cohen, Lane, LIR/NIAID; Masur, Kovacs, CC/NIAID; Nussenblatt, NEI; Salzman, Dewar, Vasudevachari, Georgetown; Driscoll, Ford, NCI; Koenig, Medimmune, Rockville, MD).

Immunologic Approaches to the Therapy of HIV Disease

An intensive effort was directed toward studying the potential therapeutic aspects of immunologic approaches to HIV infection. A study evaluating the immunologic and antiviral effects of repeated administration of IL-2 and nucleoside analogues was continued. IL-2 therapy resulted in sustained increases in numbers of CD4 cells, decreased expression of activation markers, and restoration of blastogenesis responses. A new plasma HIV RNA detection assay was employed; this revealed transient and consistent increases in viral load at the end of each infusion. In follow-up of these findings, a randomized controlled trial comparing IL-2 plus nucleoside analogues to nucleosides alone was initiated. Adoptive immunotherapy using transfers of activated and expanded syngeneic polyclonal lymphocytes was begun. Marked but transient increases in CD4 counts have been seen in the post transfer period, sometimes in association with increased viral replication in plasma. A phase I trial administering a nef-specific cytotoxic T lymphocyte clone to its donor was continued; no clinical benefit was demonstrated, and this patient's immune function has steadily declined. A protocol evaluating the safety and pharmacokinetics of NAC was modified to examine long term safety and antiviral activity.

Systemic bioavailability with oral NAC was too low to make oral dosing practical; intravenous NAC was intolerable at doses that achieve concentrations shown to be inhibitory *in vitro*. No antiviral effects were seen with up to one year of oral therapy. A study generating random recombinatorial libraries of human immunoglobulin genes from HIV infected individuals was continued. Protocols using recombinant gp160 and p24 immunization of seropositive patients were concluded; both proteins were found to be safe, but no immunologic or antiviral benefit was seen. (Walker, Fauci, Davey, Falloon, Polis, Lane, LIR/NIAID; Masur, Kovacs, CC/NIH; Schnittman, MTB/DAIDS/NIAID; Koenig, Medimmune; Burton, Scripps)

International Studies on the Acquired Immunodeficiency Syndrome

AIDS has become a global pandemic with over 13 million HIV-infected individuals worldwide. A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In one study in Kinshasa, Zaire, we demonstrated an annual seroincidence rate of 10% among female prostitutes. HIV seroconversion was strongly associated with prior infection with non-ulcerative sexually transmitted diseases (STDs) including chlamydia, gonorrhea, and trichomoniasis as well as genital ulcerations such as those caused by syphilis and chancroid. With escalating rates of HIV infection in Asia, we have also instituted a cohort study of prostitutes and patients attending STD clinics. Over the past 3 years, seroprevalence among commercial sex workers in India has risen from 7.3% to 38.6%, and among STD clinic attendees from 1% to 15%. Furthermore, we demonstrated a marked increase in HIV infection from 0.2% to 5.5% among patients attending STD clinics in Baltimore. Recent infection with syphilis, herpes and chlamydia were again strongly associated with HIV infection emphasizing the importance of STDs in HIV transmission. In our perinatal studies, we documented a 28% perinatal transmission rate which correlated with low maternal $CD4^+$ lymphocyte counts, elevated $CD8^+$ lymphocyte counts, anemia, chorioamnionitis and funisitis. We were unable to document any association between the presence or absence of neutralizing antibodies to the V3 loop and perinatal transmission. We developed a laboratory algorithm using the immune complex dissociated (ICD)-p24 antigen assay and the IgA assay that could reliably diagnose HIV infection among infected children after the first week of life with a sensitivity and specificity of 99%, respectively. In a recent survey of patients attending an inner-city emergency department, we documented an increase in HIV prevalence from 6.0% to 11.3% during a four-year period. The most significant increases were among young women, black men, heterosexuals and those without insurance. Finally, we demonstrated that individuals co-infected with HIV-1 and HTLV-1 have elevated mean CD4 lymphocyte counts despite more advanced stages of HIV disease, elevated β-2 microglobulin and p24 antigen levels. These data support the hypothesis that HTLV-1 infection accelerates the clinical course of HIV infection despite modulating an increase in non-functional $CD4^+$ lymphocytes. (Quinn, Fauci, C. Brown, LIR/NIAID; Rompalo, Zenilman, Halsey, Kelen, Johns Hopkins, Baltimore, MD)

The Genetic Response of Primary T lymphocytes to an Activation Signal: The Role of NF-κB, its Regulation and Function.

NF-κB is critically involved in the regulation of a large number of genes important for an immune response, inflammation and acute phase reactions. In addition, this factor is essential for the regulated expression of HIV as well as other viruses. NF-κB is actually a family of complexes which are normally dormant, being repressed by the inhibitory protein IκB which keeps these complexes in the cytoplasm. Many cellular activation signals, including cytokines, mitogens and stress can cause the activation of NF-κB and its translocation to the nucleus.

We have now shown for the first time that this activation of NF-κB involves the rapid proteolytic degradation of IκB, thereby freeing NF-κB for translocation into the nucleus. We also were able to demonstrate for the first time that NF-κB, once in the nucleus induces large amounts of IκB mRNA, leading to resynthesis of IκB within 40 minutes after activation. The newly synthesized IκB could be shown to reinhibit NF-κB, especially in the absence of continued signalling, thus acting to limit the activation phase and establishing an autoregulatory network for NF-κB. This mechanism suggests a strong need to downregulate initial activation events in cells.

We have demonstrated for the first time that the oncoprotein Bcl-3, which is structurally related to IκB, can participate in positive transactivation, rather than acting in an inhibitory function. IκB and Bcl-3 share similar ankyrin repeat domains, but are otherwise very different. p50B homodimers can bind to Bcl-3 on DNA and this ternary complex is capable of efficient transactivation related to transactivation domains present on Bcl-3. Bcl-3 has two cooperating transactivation domains which lie outside the ankyrin domain. p50B-Bcl-3 complexes can be detected in some highly differentiated cells where they may constitute an IκB- and signal-independent pathway for κB-dependent gene transcription.

Finally we have developed a model by which Bcl-3 also acts to facilitate transactivation of certain κB sites during cellular stimulation of most cells. p50 homodimers, which do not transactivate on their own, could be shown to bind very stably to certain κB sites and prevent access to these sites for NF-κB. Such p50 homodimers are abundant in nuclei of unstimulated primary cells, where they presumably function to repress κB sites. Bcl-3 is induced upon activation of such cells and we could show that it can enter the nucleus. Bcl-3 can now remove stably bound p50 homodimers and thus allow binding and transactivation by NF-κB. (Siebenlist, K. Brown, Franzoso, Kanno, Bressler, Fauci, LIR/NIAID; Kelly, LP, NCI; Woychik, Oak Ridge National Laboratory)

Immunoregulation of Human Lymphocyte Function

Studies are directed at understanding the regulation of human B lymphocytes at a molecular and cellular level. The cellular studies are focused on identifying B cell subsets including memory B cells and on assessing the functional capacity of these different B cells. In conjunction with these studies we have established methodology for identifying new genes that are differentially expressed among these subsets and may account for some of their functional differences. These studies are relevant to our understanding of the B cell response to vaccines and may provide means for augmenting or inhibiting these responses.

We have continued our studies of several B cell specific genes that we had previously isolated. CD22 has been shown to be a cell adhesion molecule which interacts with a broadly expressed cell surface molecule. Current efforts are directed at understanding the mechanisms by which CD22 signals B cells. Several intracellular proteins including a protein kinase have been found which interact with the intracytoplasmic portion of CD22. The identification of these proteins is in progress and should provide insights into CD22 signalling and the role of CD22 in B cell function. Our studies of the BL11 gene have continued and cell lines which constitutively express BL11 have been made. BL11 has been successfully expressed in COS cells. In addition a fusion protein between BL11 and the Fc portion of Ig is being made for further functional studies including an attempt to identify a BL11 ligand. The human BL34 protein has been identified to be a member of the helix-loop-helix (HLH) family of proteins and to be present in the nucleus of

COS transiently transfected with a BL34 expression vector. Recombinant BL34 has been purified from bacteria and anti-peptide and anti-recombinant BL34 antibodies have been made. The genomic structure of BL34 is being determined and the chromosomal location has been mapped to chromosome 1. A murine equivalent of BL34 has been identified and in this process evidence found for other BL34 related genes. Our current studies are aimed at understanding the functional importance of this interesting gene in B cells.

The mechanisms of B lineage specific expression of the CD20, CD22, and CD19 genes continued to be studied. Several transcriptional factors have been identified to be important in the genetic control of CD20 expression in B cells including Oct-2, BSAP, NF-Y, and several unknown proteins. Current studies are directed at determining the relative importance of each of these factors and identifying other genetic regulatory regions in the CD20 gene. The genomic structure of the CD22 gene has been determined and two distinct regions for the initiation of CD22 RNA transcription have been mapped which result in alternative usage of exon 1. The relative importance of these start sites and identification of regulatory elements in the CD22 gene are in progress. The proximal portion of the CD19 promoter has been studied and the regulatory cis-elements are under study. A panel of cell lines deficient in CD19 have been made and the genetic mechanisms which account for this deficiency are under investigation. These studies should lead to a better understanding of the cis-elements and transcriptional factors important in B lineage specificity and in the control of B cell differentiation.

Two human homeobox containing genes HLX and HB9 first identified in activated B lymphocytes have been found to be expressed in other hematopoietic cells including high levels in hematopoietic progenitors. Functional studies are in progress to assess the role of these genes in hematopoiesis and lymphocyte activation. Transfection and transgenic mice studies for the analysis of the HLX gene have implicated HLX as an important regulator of gene transcription in hematopoietic progenitors and in lymphocytes. Recombinant HLX protein is being used for functional studies and to determine whether proteins exist which specifically interact with HLX. A target sequence, AATTAGG for the HLX protein has been determined and the HLX gene has been shown to transactivate its own promoter. We have recently completed sequencing DNAs containing the genomic loci of the HLX and the HB9 genes. This allowed us to complete the protein coding sequence of the HB9 gene. A chimeric gene from the HB9 cDNA and the HB9 genomic clone was made and has been used for transfection studies and will be used for the production of HB9 transgenic mice. We plan to target the HB9 gene to various lymphoid cell types. Once these mice are established they will bred with the HLX transgenic mice to produce double transgenic mice.

These studies will result in a better understanding of normal B cell function. As our knowledge of basic B cell physiology increases we should gain insights into the mechanisms of abnormal B cell function which often accompanies autoimmune diseases, develop methodology for manipulating antibody responses in these diseases, and means of augmenting B cell responses to human pathogens. (Deguchi, Druey, Kang, Riva, Thevenin, Tuscano, Fauci, Kehrl, LIR/NIAID; Coligan, BRB/NIAID; Najfeld, Mt. Sinai, NY; Katz, Georgetown; Tedder, Harvard; Deguchi, Toyoma Univ., Japan; Rieckmann, Germany).

Clinical Immunopathogenic, and Therapeutic Studies of The Systemic Vasculitides and Related Disorders

Over the past 25 years we have developed therapeutic regimens which have resulted in the induction of prolonged remissions and cures in certain of the systemic vasculitides, particularly Wegener's granulomatosis. However, because of the relative high frequency of long term toxicity of this regimen, we have sought to explore the possibility of using alternative regimens.

We have continued to use and modify our computerized data bank to analyze an extensive collection of epidemiological, clinical and laboratory information about the systemic vasculitides. We have analyzed data on 60 patients with Takayasu's arteritis seen over a 24 year period and defined the clinical profile and therapeutic outcome of patients with this disease. At least 60% of patients with active disease respond to corticosteroid therapy. In patients refractory to corticosteroid therapy, 40% were able to achieve remission with the addition of a cytotoxic agent. However, 45% of patients achieving remission experienced one or more relapses. Our data indicate that current therapeutic modalities may control disease activity but are potentially toxic and do not prevent disease relapse. Using information in this data bank, we have also retrospectively reviewed the genitourinary morbidity of cyclophosphamide therapy in patients with systemic vasculitis. We have continued our study of the efficacy and safety of low dose methotrexate (MTX) in treating Wegener's granulomatosis. At present, follow-up has been adequate to analyze the outcome of 47 patients. The mean duration of follow-up is 19 months. Weekly administration of MTX and corticosteroids resulted in marked improvement in 76% of patients. Sixty four percent of patients were able to achieve remission and have their corticosteroid dose tapered to alternate day therapy or discontinued. A total of 14 patients experienced disease progression while on MTX; three patients experienced MTX toxicity requiring discontinuation of the drug; and there were 3 deaths on therapy. Thus, the total failure rate (disease progression, toxicity, death) was 40%. These results indicate that MTX may be a feasible alternative to daily cyclophosphamide in patients whose illness is not immediately life-threatening or in whom prior cyclophosphamide therapy produced significant toxicity. (Sneller, Hoffman, Leavitt, Kerr, Talar-Williams, Fauci, LIR/NIAID; Travis, NCI; Hallahan, OSD/NIAID)

Immunopathogenesis of *Chlamydia trachomatis* Infections

Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop improved molecular diagnostic assays and to examine the pathogenesis of chlamydial infections in experimental animal models and in patients with genital, ocular and respiratory infections. Utilizing a variety of diagnostic assays, *C. trachomatis* genital infections continue to be highly prevalent among patients attending STD clinics with rates of 15%-20%. We have been able to demonstrate a marked increase in sensitivity utilizing PCR, compared to culture in identifying patients with chlamydial genital infection. PCR was positive in 75% of male partners of infected females as compared to 39% by culture. Sequence analysis of PCR amplified DNA demonstrated the same sequence in paired sexual partners illustrating the accuracy and validity of PCR. We demonstrated that PCR could also be used to identify chlamydial infections in children with trachoma and to monitor response to therapy. With PCR amplification, it was evident that nearly 50% of treated children had a relapse as early as four weeks post-therapy. In an evaluation of a new molecular assay called ligase chain reaction (LCR), we demonstrated a marked increase compared to culture in the frequency of infection of genital chlamydial infections among patients attending STD clinics. We also developed a new serologic technique for the measurement of antibodies to major outer membrane protein (MOMP) of *C. trachomatis* by PCR amplification of the open reading frame of MOMP and DNA transcription to mRNA followed by translation to MOMP protein in a reticulocyte lysate system. Production of MOMP protein was then used in a serologic assay which demonstrated a serologic rise in antibodies in MOMP-vaccinated primates, which correlated with decreased infectious load. This transcription-translation radioimmunoassay may help to further analyze the immune response to MOMP and other chlamydial antigens during vaccine trials. Finally, in over 700 evaluated patients, our newly developed PCR detected *C. pneumoniae* in 8% of patients with respiratory disease and in no asymptomatic healthy controls. Additional studies utilizing PCR are planned to further describe the clinical features and transmission of both *C. trachomatis* and *C. pneumoniae* in selected populations at risk. (Quinn, LIR/NIAID; Gaydos, Bobo Viscidi, West, Zenilman, Johns Hopkins, Baltimore, MD)

Honors, Awards, and Scientific Recognition

LIR investigators have received a number of awards and honors, sit on a number of Editorial Boards, and have delivered several important scientific lectures.

Dr. Fauci serves on a number of committees of scientific and administrative importance such as the Institute of Medicine Roundtable for the Development of Drugs and Vaccines against AIDS. He is chairman of the NIH AIDS Executive Task Force as well as a member of the U.S. Public Health Service AIDS Task Force and the PHS AIDS Executive Committee. He is co-chairman of the subgroups on Therapeutic Intervention and on Vaccine of the PHS AIDS Executive Committee.

This past year, Dr. Fauci continued his role as an advisory editor of *The Journal of Experimental Medicine* and on the Editorial Board of *The Journal of Immunopharmacology, Clinics in Immunology and Allergy, EOS, Cellular Immunology, The Journal of Molecular and Cellular Immunology, AIDS Research and Human Retroviruses*, and *AIDS Patient Care*. He serves on the Advisory Board of *Immunopharmacology and Immunotoxicology, Clinical Immunology and Immunopathology*, and the *Journal of Clinical Immunology*. He is on the Editorial Advisory Council of *La Ricerca in Clinica e in Laboratorio*. He also continued his duties as associate editor of the *Journal of the Acquired Immunodeficiency Syndrome*.

He is still an editor of "Harrison's Principles of Internal Medicine," "Transactions of the Association of American Physicians," "Current Therapy in Allergy, Immunology and Rheumatology," and "Harrison's Principles of Internal Medicine - Companion Handbook." He is also an associate editor of "AIDS, Etiology, Diagnosis, Treatment, and Prevention" and "Current Therapy in Internal Medicine." In addition, he is an editor along with Dr. John I. Gallin of "Advances in Host Defense Mechanisms."

Dr. Fauci received Doctor of Sciences, Honoris Causa, degrees from the Medical College of Wisconsin, Bard College, and Bates College.

He was elected as a Fellow of the American Association for the Advancement of Science.

During the past year, Dr. Fauci delivered several prestigious lectures including the Jules Bordet Lecture at the Third International Conference on Drug Research in Immunologic and Infectious Diseases, the 1992 Ham-Wasserman Lecture of the American Society of Hematology, and the R. E. Dyer Lecture at the National Institutes of Health. He was also a keynote speaker at the Seventh International Symposium on Infections in the Immunocompromised Host, the Symposium on Human Lymphocyte Markers and Function in Health and Disease in Genoa, Italy, the Septieme Colloque des Cent Gardes in Marnes-la-Coquette, France, the National Meeting of the Society for Leukocyte Biology, the Gladstone Institute for Virology and Immunology Inauguration Seminar, the Vincent du Vigneaud Memorial Research Symposium, the Alpha Omega Alpha Lecture of the University of Oklahoma Medical School, Distinguished Scientists Seminar Lecture at Howard University

Dr. Fauci was an invited symposium speaker at the American Association of Immunologists meeting and the International Congress on Immunology in Budapest, where he also chaired a symposium. He delivered the state of the art lecture at the International

Conference on AIDS/IIIrd World Congress on STDs in Amsterdam, the keynote address at the International Conference on AIDS Education. In addition, Dr. Fauci was an invited seminar speaker at The Johns Hopkins University Immunology Council, plenary speaker, at the Clinical Immunology Society meeting, a lecturer for the Harvard AIDS Institute Keynote Speaker Series, a Rockefeller University Colloquim Speaker, and a special lecturer at the Symposium on Lymphocyte Subpopulations in Santa Margherita Ligure, Italy.

He delivered commencement addresses at the Medical College of Wisconsin, Bard College, and Stanford University School of Medicine.

Dr. Fauci received the following awards: the Cornell University Medical College Alumni Award of Distinction, honorary title of "Il Commendatore della Repubblica" from the President of the Republic of Italy, the Thomas H. Ham - Louis R. Wasserman Award of the American Society of Hematology, the R. E. Dyer Lectureship Award of the National Institutes of Health, the DHHS Secretary's Certificate of Appreciation for "Outstanding contribution in the implementation of the parallel track initiative of the U. S. Department of Health and Human Services," the 1992 Dr. Nathan Davis Award for Outstanding Public Service from the American Medical Association, and the Howard University Outstanding Achievement Award. In addition, his article - Lane, Masur, Edgar, Whalen, Rook, and Fauci: Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* 309: 453, 1983 - was listed in the 1993 Citation Classics (Current Contents).

Dr. Guido Poli received the 1992 NIH Award of Merit. In addition, he was invited to speak at several lectures including the FERS/EFAR Meeting "New Concepts in AIDS Pathogenesis", Annecy (Less Pensieres), France June 11-12 1992, "Cytokines and HIV Expression". The Johns Hopkins University Immunology Council, Baltimore, MD, November 3, 1992, "Cytokines and Their Effects on HIV". "Free Radicals and Antioxidants in AIDS and HIV Infections", CERLIB, Less 2-Alpes Francs, Grenoble, January 22-23, 1993 "Role of UV Radiation and Cytokines in HIV Replication". And at the International Conference on "HIV and AIDS. Society of Chemical Industry, London, SW1, 20th/21st May 1993. "The Experimental Basis for Cytokines and Anti-Cytokine Therapy in HIV Infection" Current Therapies - Future Directions".

Dr. Andrew Dayton chaired a session of tat/rev at the 1993 Cold Spring Harbor Meeting on Retroviruses. Dr. John Kehrl was elected to the American Society for Clinical Investigation and joined the editorial board of BLOOD. Dr. Sharilyn Stanley received a PHS Commendation Medal. Dr. David Cohen presented a lecture at the Washington International Spring Symposium, "The Cell Cycle," with accompanying invitation to submit a manuscript to the meeting Proceedings.

Dr. Judith Falloon organized several conferences including the Combined Clinical Staff Conference, February, 26, 1992, The National Institutes of Health, "Recent Advances in the Management of AIDS-Related Opportunistic Infections". H. Masur, Chair, Moderator, H. Clifford Lane; Discussants: B.E. Laughon, J. Falloon, J.A. Kovacs, R.T. Davey, Jr., M.A. Polis, H. Masur. And the U.S. Public Health Service Task Force Conference on Prophylaxis and Therapy for Mycobacterium avium complex. Recommendations on prophylaxis and therapy for disseminated Mycobacterium avium complex for adults and adolescents infected with human immunodeficiency virus.

Administrative, Organization and Other Changes

The major theme of the LIR continues to be focused on studies of the immunopathogenesis and therapy of human immunodeficiency virus (HIV) disease and the acquired immunodeficiency disease (AIDS). In addition, the laboratory studies the cellular and molecular mechanisms of the regulation of the human immune system in normal and disease states in addition to HIV disease. The size of the LIR in space, slots, and resources has been stable over the past few years.

The immunopathogenesis section of the LIR moved to the A Wing of Building 10 this year. Drs. Gary Hoffman and Randi Y. Leavitt of the Section of Vasculitis and Related Disorders left the NIH after 6 and 12 years respectively. Dr. Michael Sneller assumed the position of Head of this Section. Ms. Cheryl Talar-Williams joined the section as a study coordinator.

Drs. Michael Granberry, Carlo Muro-Cacho and Yuexia Li joined the LIR this year. Dr. Christopher Brown left the LIR to the practice of Infectious Diseases and Internal Medicine in Wyoming. Dr. Mauro Vaccarezza of Genoa, Italy joined the laboratory as a guest researcher and Dr. Luca Butini returned to Verona, Italy after a very successful fellowship at LIR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00675-01 LIR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Lymphoid Tissue in the Pathogenesis of HIV-1 Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	G. Pantaleo	Visiting Scientist	LIR, NIAID
	C. Graziosi	Visiting Scientist	LIR, NIAID
	C. Muro-Cacho	Visiting Associate	LIR, NIAID
	O.J. Cohen	Medical Staff Fellow	LIR, NIAID
	M. Vaccarezza	Visiting Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*
Dept. Pathol. GWU (J. Orenstein); Molecular Histolabs (C. Fox); PRI, Frederick, MD (M. Baseler); St. Luke's Hosp., NYC (D. Kotler); Univ. Alabama, Birmingham, AL (G Shaw, M. Saag); Montreal (R. Sekaly); Univ. Acoro (L. Butini, M. Montroni).

LAB/BRANCH
Laboratory of Immunoregulation

SECTION
Immunopathogenesis Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have demonstrated that lymphoid organs function as a major reservoir for human human immunodeficiency virus type 1 (HIV-1). We have demonstrated that during clinical latency, HIV accumulates in the lymphoid organs and replicates actively despite a low viral burden in peripheral blood. The histopathologic abnormalities are responsible for trapping of viral particles and sequestration of HIV-1 infected cells. Viral particles are trapped within the follicular dendritic cell (FDC) network of the germinal center. A progressive disruption of lymphoid tissue architecture is associated with disease progression. Efficient trapping of virus corresponds directly with the integrity of the FDC network. Viral burden and viral replication tend to equilibrate in late stage disease. A state of true microbiological latency does not exist during the course of HIV-1 infection. Peripheral blood does not accurately reflect the actual state of HIV disease. HIV disease is active and progressive during clinical latency. We have investigated virus expression and distribution in the gut associated lymphoid tissue. We have demonstrated trapping of virus in the FDC network associated with the lymphoglandular complexes disseminated in the gut. Rarely, isolated cells expressing HIV-1 RNA have bene observed in the gut. Similar levels of viral burden and replication have been observed in the gut. Similar levels of viral burden and replication have been found in mononuclear cells isolated from peripheral blood and bronchoalveolar lavage (BAL). We have investigated cytokine expression in peripheral blood and lymphoid tissues from the same patients and have demonstrated a decline in the levels of expression of interferon-gamma and interleukin-2 (IL-2) in both peripheral blood and lymphoid tissue associated with disease progression. Levels of expression of TNF-alpha and IL-10 did not change in the different stages of disease. Levels of expression of IL-6 increased in peripheral blood with disease progression. IL-4 was never expressed in both peripheral blood and lymphoid tissue in any stage of disease. Immunopathogenic insights gained from these studies will have important implications in the design of therapeutic strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00676-01 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenic Mechanisms of HIV Infection: The Role of Cellular Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. Pantaleo	Visiting Scientist	IR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	C. Graziosi	Visiting Scientist	LIR, NIAID
	C. Muro-Cacho	Visiting Associate	LIR, NIAID
	O.J. Cohen	Medical Staff Fellow	LIR, NIAID
	M. Vaccarezza	Visiting Fellow	LIR, NIAID
	D. Weissman	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

Dept. Pathol. GWU (J. Orenstein); Molecular Histolabs (C. Fox); PRI, Frederick, MD (M. Baseler); Ctr. Blood Res. and Harvard Med. Sch., Boston (T. Springer, A. deFougerolles); Univ. Montreal (R.P. Sekaly); Dana Farber (Tedder)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have investigated the immunopathogenic mechanisms responsible for CD4+ T cell depletion. Apoptosis has never been observed in mononuclear cells freshly isolated from peripheral blood. Evidence for apoptosis has been observed in lymph node sections from HIV infection individuals. Macrophages containing apoptotic bodies have been observed in lymphoid tissue sections. Bone marrow derived dendritic cells (DC) are comprised of 2 populations only one of which is infectable by HIV. This latter population may in fact be a monocyte lineage cell. DC can bind HIV and pass infection on to unstimulated CD4+ T cells. We have demonstrated that the HLA complex influences the expressed T-cell receptor repertoire. Significant differences in the expression of the Vβ repertoire have been observed in CD4+ and CD8+ T cell subsets. We have demonstrated that distinct sets of Vβ families are perturbed in circulating CD4+ T cells in HIV infected individuals. These results may be consistent with the presence of a superantigen. LFA-1/ICAM-1-2-3 pathway of cell-to-cell adhesion regulates HIV-mediated syncytia formation. ICAM-1, ICAM-2 and ICAM03 molecules function as counter-receptors for LFA-1 in the process of HIV-mediated syncytia formation. Cyclosporine A (CsA) exerts a significant inhibition (60-80%) on HIV infection *in vitro* at concentrations corresponding to easily tolerated serum levels of CsA in the treatment of other diseases. Suboptimal concentrations of CsA and AZT cooperate in suppressing HIV-1 infection *in vitro*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00677-01 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Cytokines In the Regulation of HIV Expression.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Guido Poli	Visiting Scientist	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	P. Biswas	Guest Researcher	LIR, NIAID
	C. Brown	Senior Staff Fellow	LIR, NIAID
	D. Weissman	Clinical Associate	LIR, NIAID
	L. Fox	Clinical Associate	LIR, NIAID
	G. Franzoso	Visiting Fellow	LIR, NIAID
	U. Siebenlist	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

LID, NIAID (V. Hirsch); Dept, Pathol. GWU (J.M. Orenstein); CABM, UMDNJ, RWMS, Piscataway, NJ (A.B. Rabson); Molecular Histology Lab Inc., Gaithersburg, MD (C. Fox).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cellular and molecular pathways involved in the regulatory effects of pro-inflammatory and immunoregulatory cytokines on human immunodeficiency virus (HIV) expression were investigated. Synergistic induction of HIV expression was demonstrated in chronically infected U1 cells co-stimulated with several cytokines. Interleukin (IL)-4 and IL-10 potentiated HIV expression of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), IL-1, and IL-6. Autocrine stimulation of HIV replication by multiple endogenous cytokines was demonstrated in primary human peripheral blood mononuclear cells without necessity of mitogen stimulation as well as in monocyte-derived macrophages. In this system, as well as in U1 cells, cytokine antagonists such as IL-1ra and pentoxifylline suppressed HIV replication. *In vivo*, macaques infected with SIV showed a peak of cytokine production coincidental with the early peak of viremia that characterizes both SIV and HIV primary infections. At the molecular level, a central role of NF-κB in HIV expression has been further demonstrated. Isogenic cell clones of the CD4(+) U937 cell line showed either high (PLUS clones) or very low (MINUS clones) ability to support HIV replication, a functional dichotomy that was correlated with the inablity of MINUS clones to support the transactivation of the viral promoter. The functional defect was found dependent upon a different pattern of NF-κB-related proteins, and specifically, with the presence of a different isoform of the p65 subunit of NF-κB. Finally, mutagenesis conducted on the p50 subunit of NF-κB resulted in a protein which maintained the ability to specfically bind to the DNA target sequences, but did not dimerize with the p65 subunit, therefore acting as a trans-dominant mutant of NF-κB-mediated transcription.

Continued

Others:	P. Bressler	Medical Officer	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00678-01 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Infection of Bone-Marrow and Thymus in the Pathogenesis of HIV Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.K. Stanley	Senior Clinical Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	M.E. Granberry	Staff Fellow	LIR, NIAID
	C. Muro-Cacho	Visiting Associate	LIR, NIAID

COOPERATING UNITS *(if any)*

Molecular Histology Lab, Inc., Gaithersburg, MD (C. Fox); Dept. Pathol. GWU (J. Orenstein); PRI, Frederick, MD (M. Baseler); Duke Univ. Med. Ctr. (B. Haynes); Animal Care Branch, NIAID (P. Golway); Systemix, Inc. (J.M.McCune).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	2.25	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at delineating the pathogenesis of human immunodeficiency virus (HIV) infection in thymus and bone marrow. We have identified *in vivo* and *in vitro* infection of CD34+ bone marrow progenitor cells. Viral burden in the bone marrow is not consistently higher than that in the peripheral blood; stromal degeneration may occur in HIV infected bone marrows. Human thymus is reproducibly infected with HIV in the SCID-hu mouse using primary isolates and is associated with infection of CD4/CD8 double positive, CD4 single positive and CD8 single positive thymocytes. Thymocyte depletion is marked and may be associated with increased apoptosis in the HIV infected thymus. Thymic epithelial cells are also productively infected with HIV and may serve as a viral reservoir in the thymus. Degeneration of thymic epithelial cells occurs independently of infection and function of these cells may be disrupted. After implantation in the scid mouse human fetal lymph node and intestine can be infected by intravenous injection of virus. HIV expression can be regulated by CD8+ T cells from asymptomatic patients, which can suppress viral replication in autologous and allogeneic CD4+ T cells by production of a soluble factor. Suppression of virus expression is not associated with decreased proliferation of CD4+ T cells. Virus expression is also regulated by cytokines, and, after ultraviolet irradiation, epidermal keratinocytes produce a factor or factors which induce the production of HIV from chronically infected U1 and ACH-2 cells. Tumor necrosis factor-α is one of the cytokines produced by irradiated epidermal keratinocyte cells which mediates this effect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00679-01 LIR

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Therapy for HIV Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Bressler	Medical Officer	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	S.K. Stanley	Senior Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

NHLBI/MHB (F. Anderson, R. Morgan)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the past few years, a number of laboratories have become involved in determining the feasibility of utilizing the molecular techniques of gene manipulation or gene therapy as part of the strategy for the treatment of human immunodeficiency virus (HIV) infection. One approach has been to transduce into peripheral blood CD4+ T cells selected genes of HIV which have been mutated and which can serve as trans-dominant mutants to block the expression of HIV if that cell ultimately became infected. Ideally, stem cells might be transduced and reinfused into the host thus providing a progeny of cells which are resistant to HIV infection and or expression. Another approach to gene therapy has been to transduce into peripheral blood cells genes for substances that when secreted or expressed would protect the cells themselves or surrounding cells from HIV infection. In this regard, we have successfully transduced, using retroviral vectors, cell lines and peripheral blood lymphocytes with a gene that expresses soluble CD4-IgG and have partially protected these cells against spreading HIV-1 infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00585-04 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenic Mechanisms in HIV and Other Retroviral Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.I. Cohen	Medical Officer	LIR, NIAID
Others:	H.C. Lane	Section Head	LIR, NIAID
	V.Kolesnitchenko	Visiting Fellow	LIR, NIAID
	H. Tian	Guest Worker	LIR, NIAID
	R. Lempicki	Staff Fellow	LIR, NIAID
	G. Scully	Clinical Associate	LIR, NIAID

COOPERATING UNITS *(if any)*

CBMB, NICHD (L. Samelson); DCT, BRMP, NCI (J. Ashwell); UA Birmingham (G. Shaw); Scripps Institute (P. Russell, C. McGowan); Georgetown University School of Medicine (D.P. Hartmann, J. Cossman); New York Univ. (S. Zolla-Pazner); U C San

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.2	4.2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Understanding the molecular mechanisms used by human immunodeficiency virus type 1 (HIV-1) to kill CD4+ T lymphocytes should critically contribute to finding new therapies capable of interrupting the progression to AIDS which follows HIV infection. Utilizing a transfection system previously developed by our laboratory employing Jurkat CD4+ T cell lines, we have defined a central biochemical mechanism by which HIV-1 kills CD4+ cells. We showed that HIV-1 induces a form of programmed cell death (PCD) triggered by tyrosine phosphorylation of cellular proteins, which leads to hyperphosphorylation of cyclin dependent kinase p34cdc2, an enzyme critically involved with the regulation and disregulation of the cell cycle at the G2/M checkpoint. We demonstrated that primary, clinical HIV-1 isolates use this mechanism ex vivo to kill CD4+ peripheral blood lymphocytes contributing to both the single cell and multicellular cytopathic effect commonly associated with pathogenic clinical variants of HIV-1. These studies employed antibodies to a G2/M phase-specific cell division cycle protein, cyclin B, to establish that cells dying of HIV cytopathicity were aberrantly arrested at the G2/M interface of the cell cycle. These antibodies can now be used to directly study T cells from HIV-infected individuals to learn 1) what population of T cells in infected individuals undergoes this killing process and 2) whether the percentage of affected cells can be used to predict progression to AIDS. We further demonstrated with a panel of metabolic inhibitors that HIV-mediated PCD substantially differed from the programmed death of CD4+ T cells which occurs during normal T cell development (negative selection). This finding has thereby increased the likelihood that clinically useful agents can be developed to selectively inhibit the HIV-killing process. Two compounds, the tyrosine kinase inhibitor, herbimycin A, and the phosphatase 2A inhibitor, okadaic acid, were found capable of blocking HIV-mediated killing, although both compounds have substantial toxicity probably limiting any clinical utility. Work continued on developing genetically engineered T cell receptors to direct cytolytic T cells against the HIV-1 envelope proteins. Retroviral vectors encoding chimeric antibody/T cell receptors recognizing either the extracellular or transmembrane HIV envelope glycoprotein were constructed and tested for function. Transgenic mice were produced which were capable of synthesizing chimeric receptor mRNA in their circulating T cell pool.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00587-04 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Gene Expression of the Human Immunodeficiency Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.I. Dayton	Sr. Staff Fellow	LIR, NIAID
Others:	E.T. Dayton	Staff Fellow	LIR, NIAID
	D.M. Powell	Graduate Student	LIR, NIAID
	R.K. Hsu	Summer Student	LIR, NIAID
	S. Lee	Clinical Associate	LIR, NIAID
	S. Bende	IRTA Fellow	LIR, NIAID
	L. Butini	Visiting Scientist	LIR, NIAID
	G. Pantaleo	Visiting Scientist	LIR, NIAID
	M.J. Zhang	Visiting Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

LMB, NCI (J.V. Maizel, B. Shapiro); U. Colorado, Boulder (D. Konings); LTCB, NCI (R. Gallo); LTCB, NCI (M. Klotman); James N. Gamble Inst., Cincinnati, OH (B. Baroudy, G. Kotwal).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have detected in crude cellular extracts a nuclear activity which cooperatively binds with the *Rev* protein to the *Rev* response element (RRE). This activity should be crucial for the *in vivo* function of *Rev*, the emergence from microbiological latency and the synthesis of mature virions during acute infection. Chromatographic analysis of the activity has determined it consists of at least four and possibly more than five separate proteins. One of the activities has been purified to the extent of five prominent bands on an SDS gel. We should soon be able to sequence and clone it. We have acquired sufficient physicochemical data about the other factors to facilitate their rapid cloning. We are now studying the factors by microinjecting them into mammalian cells along with *Rev*-dependent indicator genes. In related work we have studied the interaction of the *Rev* protein with the RRE. We have found that RRE elements determine the binding of *Rev* molecules subsequent to the first binding event. This precludes models for *Rev*/RRE binding which hold that all of the specificity is in the first binding event and that subsequent multimerization of *Rev* along an RNA molecule involves only non-specific recognition of RNA by *Rev* protein. Along slightly different lines, we have used subtractive cloning techniques to isolate clones of a number of cellular genes specifically up- and down-regulated by HIV infection. Surprisingly, HIV infection turns on Line-1, an endogenous human retroviral element, and switches off expression of S16 ribsomal protein. The mechanism and significance of these findings is currently being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00634-02 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigation of the SCID-hu-PBL Mouse Model of HIV-1 Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Davey Jr.	Senior Investigator	LIR, NIAID
Others:	H.C. Lane	Section Chief	LIR, NIAID
	K. Ruxrungtham	Visiting Scientist	LIR, NIAID
	M. Boyle	Visiting Scientist	LIR, NIAID
	B. Smith	Guest Worker	LIR, NIAID
	D. Cohen	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

Laboratory of Medicinal Chemistry, National Cancer Institute (John S. Driscoll, Harry Ford Jr.); MedImmune, Inc. (Scott Koenig)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	2.7	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Based upon an analysis of three different experimental murine animal models of human immunodeficiency virus type 1 (HIV-1) infection in SCID mice, we determined that one model was particularly well-suited to adaptation in the testing of putative antiretroviral agents. Both in terms of reliability and technical feasibility, the SCID-hu-PBL model (involving reconstitution of 8-12 week old SCID mice with fresh peripheral blood mononuclear cells obtained by lymphapheresis from human donors) was selected to test the comparative antiretroviral activity of a new nucleoside analogue, 2--ß-Fluoro-dideoxyadenosine (FddA), relative to zidovudine. Reconstitution of SCID mice by intraperitoneal injection of harvested mononuclear cells led to successful and sustained transfer of lymphocytes that were optimally infectable 2-3 weeks after reconstitution. Human Ig antibody production was demonstrated in reconstituted mice as was trafficking of a small percentage of human lymphocytes to the murine spleen. The optimal titer of infectious HIV-1 IIIB was determined using viral detection by both lymphocyte co-cultivation and polymerase chain reaction methods. In a series of dose-ranging experiments, oral and intraperitoneally-administered FddA demonstrated potent dose-dependent suppression of HIV-1 infection consistently superior to zidovudine in this model. The kinetics of the protective effects of FddA pre- and post-exposure to HIV-1 were explored and revealed that FddA may also have significant chemoprophylactic utility in this regard. In another series of experiments, the adoptive transfer of protective immunity using peripheral blood mononuclear cells and plasma from gp160-immunized donors was studied. No protective effect of the gp160-primed lymphocytes or high-titer plasma could be demonstrated in the SCID-hu-PBL system. Currently, this model is also being adapted for the testing of other putative antiretroviral agents (including a monoclonal antibody directed against the V3 loop of gp120) as well as for an assessment of the relative degree to which infected lymphocytes are resistant to secondary infection by different strains of HIV-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00390-10 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Anti-Retroviral Therapy of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Polis	Senior Investigator	LIR, NIAID
Others:	H.C. Lane	Section Head	LIR, NIAID
	R.T. Davey	Senior Investigator	LIR, NIAID
	R.E. Walker	Senior Investigator	LIR, NIAID
	J. Falloon	Senior Investigator	LIR, NIAID
	E.A. Siedel	Medical Officer	LIR, NIAID

COOPERATING UNITS *(if any)*

CC, NIH (H. Masur, J.A. Kovacs); NEI, NIH (R. Nussenblatt); Georgetown University (N. Salzman, R. Dewar, M. Vasudevachai)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.1	2.1	2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed toward studying the preventive and therapeutic aspects of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS). A randomized trial comparing therapy with zidovudine (ZDV) versus interferon-alpha (IFN-α) versus the combination in 180 patients with early HIV-1 infection completed accrual and is currently active. A dose-tolerance study of the combination of IFN-α and dideoxyinosine (ddI) completed accrual of 53 patients and is now gathering long-term follow-up data. A randomized, double-blinded, placebo-controlled Phase II study of L-697,661 versus ZDV completed accrual of 84 patients. In this study, plasma viremia was found to be a reliable surrogate for anti-viral activity and the early development of resistance was noted using surrogate markers as efficacy parameters. A randomized, dose escalation study of U-90152S in combination with ZDV and ddI versus ZDV and ddI alone has enrolled 18 patients and continues to enroll. A dose-escalation study of PMEA has enrolled 12 patients and continues to enroll at the second dose level. Early results suggest that this agent has anti-retroviral activity in vivo. A phase I study of CD4-Pseudomonas Exotoxin (sCD4-PE40) in HIV-infected patients was initiated and enrolled 24 patients to determine the pharmacokinetics, maximally tolerated dose, and preliminary efficacy of this agent. Follow-up analysis of a randomized, controlled, masked trial of foscarnet in the treatment of cytomegalovirus retinitis in AIDS patients demonstrated an apparent survival benefit of this therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00635-02 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Trials for the Prevention and Treatment of HIV-Associated Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Falloon, M.D.	Senior Investigator	LIR, NIAID
Others:	H.C. Lane	Section Head	LIR, NIAID
	R. Davey, Jr.	Senior Investigator	LIR, NIAID
	M. Polis	Senior Investigator	LIR, NIAID
	R. Walker	Senior Investigator	LIR, NIAID
	M. Sneller	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

CC, NIH (H. Masur, J. Kovacs, K. Spooner, B. Stearn, B. Baird, D. O'Neill, G. Kelly, M. Larson, M. Amantea), PRI (M. Baseler), NCI (M. Turner)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort directed at improving the prevention and treatment of acquired immunodeficiency syndrome (AIDS)-associated opportunistic infections continued. Cytomegalovirus (CMV)-IG (Cytogam) did not decrease CMV viruria in treated patients and was shown to be unlikely to be of benefit as prophylaxis against disease caused by CMV. Evaluation of HPMPC, a nucleotide analog, for possible use as in the prevention or treatment of CMV disease continues after finding significant nephrotoxicity at higher doses but a long duration of activity possibly permitting infrequent dosing with concomitant probenecid to decrease toxicity. An on-going blinded Phase I/II study of sparfloxacin, azithromycin, and the combination will provide safety, pharmacokinetics, and efficacy assessment of these agents for the treatment of M. avium complex infection; a salvage regimen will assess these agents plus ethambutol and clofazimine. A safety and pharmacokinetic assessment of levofloxacin, a quinolone with potential utility in the treatment of tuberculosis is planned. Studies on atovaquone (BW566C80) continue, and the efficacy and pharmacokinetics of a new suspension formulation is being assessed for use in the treatment and prevention of pneumocystis pneumonia. We have shown that the addition of pyrimethamine to dapsone for weekly use for the prevention of pneumocystis pneumonia does not change the pharmacokinetics of dapsone. An assessment of atovaquone plus pyrimethamine for the treatment of cerebral toxoplasmosis continues. Atovaquone was not effective for the treatment of cryptosporidiosis or microsporidiosis. The role of Demodex, a mite, in AIDS-associated dermatologic disease is being assessed. The epidemiology and clinical and immunological manifestations of patients with idiopathic CD4+ T-lymphocytopenia were assessed; the disorder is likely to be multifactorial in origin and no evidence of a retroviral etiology was found.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00636-02 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Approaches to the Therapy of HIV-1 Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Walker	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	H.C. Lane	Section Chief	LIR, NIAID
	R.T. Davey, Jr.	Senior Investigator	LIR, NIAID
	J. Falloon	Senior Investigator	LIR, NIAID
	M. Polis	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

CC, NIH (H. Masur, J. Kovacs); DAIDS, MTB (S. Schnittman) Medimmune (S. Koenig) Scripps Institute (D. Burton)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed toward studying the potential therapeutic aspects of immunologic approaches to human immunodeficiency virus (HIV) infection. A study evaluating the immunologic and antiviral effects of repeated administration of interleukin-2 (IL-2) and nucleoside analogues was continued. IL-2 therapy resulted in sustained increases in numbers of CD4 cells, decreased expression of activation markers, and restoration of blastogenesis responses. A new plasma HIV RNA detection assay was employed; this revealed transient and consistent increases in viral load at the end of each infusion. In follow up of these findings, a randomized controlled trial comparing IL-2 plus nucleoside analogues to nucleosides alone was initiated. Adoptive immunotherapy using transfers of activated and expanded syngeneic polyclonal lymphocytes was begun. Marked but transient increases in CD4 counts have been seen in the post transfer period, sometimes in association with increased viral replication in plasma. A phase I trial administering a nef-specific CTL clone to its donor was continued; no clinical benefit was demonstrated, and this patient's immune function has steadily declined. A protocol evaluating the safety and pharmacokinetics of N-acetylcysteine (NAC) was modified to examine long term safety and antiviral activity. Systemic bioavailability with oral NAC was too low to make oral dosing practical; intravenous NAC was intolerable at doses that achieve concentrations shown to be inhibitory in vitro. No antiviral effects were seen with up to one year of oral therapy. A study generating random recombinatorial libraries of human immunoglobulin genes from HIV infected individuals was continued. Protocols using recombinant gp160 and p24 immunization of seropositive patients were concluded; both proteins were found to be safe, but no immunologic or antiviral benefit was seen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00361-11 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

International Studies on the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	C. Brown	Expert	LIR, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins University (A. Rompalo, J. Zenilman, N. Halsey, G. Kelen).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIH, NIAID, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The acquired immunodeficiency syndrome (AIDS) is a global pandemic with over 13 million human immunodeficiency virus (HIV)-infected individuals worldwide. A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In Zaire we demonstrated a 40% infection rate in female prostitutes with an estimated 10% annual seroincidence. In India we documented a rise in HIV infection among prostitutes from 7.3% to 38.6% and from 1% to 15% among STD clinic attendees over the past 3 years. Among patients attending STD clinics in Baltimore, a retrospective 10-year survey demonstrated a marked increase in HIV infection from 0.2 to 5.5%. Common to all three cohorts was the strong association of HIV infection among heterosexuals with recent genital ulcerative and non-ulcerative sexually transmitted diseases. Even among patients attending a U.S. inner-city emergency department, HIV infection increased from 6.0% to 11.3% over 4 years with a marked increase among young women, black males, and heterosexuals. In perinatal studies of over 500 children born to HIV-infected women, we documented a 28% perinatal transmission rate which correlated with depressed maternal CD4+ and elevated CD8+ lymphocyte counts, anemia, chorioamnionitis and funisitis. There was no association between the presence of neutralizing antibodies of the V3 loop of gp120 and perinatal transmission. We developed an algorithm utilizing two serologic assays, the immune complex dissociated-p24 antigen assay and the IgA assay, which could reliably diagnose perinatal infection after the first week of life with a sensitivity and specificity of 99%, respectively. In two separate cohorts in Brazil and Haiti, we documented that human T lymphotrophic virus type 1 (HTLV-1) infection may accelerate the clinical course of HIV infection despite modulating the increase in nonfunctional CD4+ lymphocytes. Additional studies are planned to further elucidate the immunologic modulation of HIV infection by co-infection with HTLV-1 infection in these cohorts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00210-13 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulation of Human Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.H. Kehrl	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	Y. Deguchi	Visiting Associate	LIR, NIAID
	K. Druey	Medical Staff Fellow	LIR, NIAID
	V. Kang	Staff Fellow	LIR, NIAID
	A. Riva	Guest Researcher	LIR, NIAID
	C. Thevenin	Visiting Associate	LIR, NIAID
	J. Tuscano	Guest Researcher	LIR, NIAID

COOPERATING UNITS *(if any)*

BRB, NIAID (J. Coligan); NIAID, NIH; Mount Sinai Medical Center, NY (V. Najfeld, Georgetown Medical Center (P. Katz); Harvard Medical School, Boston, MA (T. Tedder) Toyoma University, Japan (Y. Deguchi); Gottingen, Germany (P. Rieckmann).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of the mechanisms which regulate the activation, proliferation and differentiation of human B lymphocytes on both a cellular and a molecular level. We have mapped the CD22 gene transcription start sites and begun to characterize the expression of this gene at a molecular level. We have provided evidence that CD22 interacts with a broadly expressed ligand and begun to study the mechanisms by which CD22 signals. We have discovered a new B cell activation gene, BL34, that encodes for a protein which is a member of the basic HLH family and may function as a transcription factor. Recombinant BL34 protein has been made and new anti-BL34 antibodies produced. The gene for BL34 has been partially delineated and its chromosomal location identified. The human BL11 gene has been transfected into several lymphoid cell lines and a fusion protein between BL11 and the constant region of IgG has been made for functional studies. The distal portion of the CD20 promoter has been studied and found to contain a BSAP binding site, a NF-Y binding site, and a third unidentified site. Evidence for other regulatory regions in the CD20 gene have been found. Cis-elemnts in the proximal portion of the CD19 promoter has been found to be very active in lymphocytes. B cell lines deficient in CD19 have been established and will be very useful for studying B cell specific gene regulation. The GC kinase gene which is differentially expressed between mantle zone and germinal center B cells has been studied in further detail. Antibodies have been made and recombinant GC kinase produced in bacteria. Preliminary evidence for kinase activity has been found. The human homeobox gene HLX has been used to generate transgenic mice using the T cell receptor promoter/enhancer and the β-actin promoter. These mice will be useful in understanding the functional consequences of HLX overexpression. A potential HLX target site has been identified as well as several potential target genes. Recombinant HLX protein has been in bacteria and is being used for functional studies and antibody production. Both the HLX gene and another human homeobox gene HB9 have been fully sequenced. Constructs for the expression HB9 have been made and transfected into various cell types. A study of one of the major transcriptional regulatory regions in the JC virus in the context of B cells has been completed. Methodology for studying differential gene expression in subsets of B cells have been established.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00431-09 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Molecular Biologic Approach to Immune Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	U. Siebenlist	Senior Investigator	LIR, NIAID
Others:	K. Brown	Visiting Associate	LIR, NIAID
	G. Franzoso	Visiting Fellow	LIR, NIAID
	T. Kanno	Visiting Associate	LIR, NIAID
	Y.-C. Lin	ERTA Fellow	LIR, NIAID
	P. Bressler	Senior Staff Fellow	LIR, NIAID
	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS *(if any)*

LP, NCI (K. Kelly); Oak Ridge National Laboratory (R. Woychik).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory has investigated the fundamental question of how the immune system is activated. In particular, we have focused on the molecular mechanisms underlying the activation of T cells, a key step during an immune response. The initial approach to gain fundamental insights into the early phase of activation of human primary T lymphocytes involved the isolation and characterization of novel mitogen- and antigen-inducible genes, including cytokines, transmembrane proteins, a nuclear phosphatase, a ras-related protein and six different transcription factors. Two of these originally cloned factors (p50 and p50B) are members of the Rel-related protein family, a family which also includes c-Rel, p65 and RelB and from which the dimeric NF-κB transcription factor complexes are generated. The NF-κB complexes are an important component of the immune response; they are essential to the regulated expression of many immunomodulatory proteins, acute phase proteins and viruses, including the human immunodefieceny virus (HIV). Upon antigen- or cytokine- or stress-mediated signalling NF-κB is released from a cytoplasmic inhibitor called IκB to translocate to the nucleus. Recently we have discovered that the activation of NF-κB involves a rapid proteolytic degradation of the inhibitory IκB protein. We have also discovered that the newly activated nuclear NF-κB induces expression of its own inhibitor, IκB, which in turn strives to restore the inhibited state again. Therefore activation is self-limiting and transient. Bcl-3 is an oncoprotein which is structurally related to IκB, but which helps to transactivate through κB sites, rather than inhibit like IκB. It can associate directly with p50B homodimers on DNA and transform these non-transactivating complexes into transactivating ones by means of activation domains present on Bcl-3. This provides a novel by-pass mechanism for activation through κB sites, independent of signalling through IκB and dependent only on expression of Bcl-3 and p50B.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00213-13 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical, Immunopathogenic, Therapeutic Studies of Vasculitis and Other Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.C. Sneller	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	G.S. Hoffman	Senior Investigator	LIR, NIAID
	R.Y. Leavitt	Senior Investigator	LIR, NIAID
	G.S. Kerr	Visiting Associate	LIR, NIAID
	C. Talar-Williams	Study Coordinator	LIR, NIAID

COOPERATING UNITS *(if any)*

NCI, NIH (W.D. Travis); NIAID, NIH (C. Hallahan).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS: 3	PROFESSIONAL: 3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued to use and modify our computerized data bank to analyze an extensive collection of epidemiological, clinical and laboratory information about the systemic vasculitides. We have analyzed data on 60 patients with Takayasu's arteritis seen over a 24 year period and defined the clinical profile and therapeutic outcome of patients with this disease. At least 60% of patients with active disease respond to corticosteroid therapy. In patients refractory to corticosteroid therapy, 40% were able to achieve remission with the addition of a cytotoxic agent. However, 45% of patients achieving remission experienced one or more relapses. Our data indicate that current therapeutic modalities may control disease activity but are potentially toxic and do not prevent disease relapse. Using information in this data bank, we have also retrospectively reviewed the genitourinary morbidity of cyclophosphamide therapy in patients with systemic vasculitis. We had established over the past 25 years that cyclophosphamide therapy is the treatment of choice for the systemic vasculitides, particularly Wegener's granulomatosis. However, in a continued attempt to determine if alternative therapies might provide comparable results without the well-known toxic side effects of cyclophosphamide, we have conducted clinical trials with other immunosuppressive agents. We have continued our study of the efficacy and safety of low dose methotrexate (MTX) in treating Wegener's granulomatosis. At present, follow-up has been adequate to analyze the outcome of 47 patients. The mean duration of follow-up is 19 months. Weekly administration of MTX and corticosteroids resulted in marked improvement in 76% of patients. Sixty four percent of patients were able to achieve remission and have their corticosteroid dose tapered to alternate day therapy or discontinued. A total of 14 patients experienced disease progression while on MTX; three patients experienced MTX toxicity requiring discontinuation of the drug; and there were 3 deaths on therapy. Thus, the total failure rate (disease progression, toxicity, death) was 40%. These results indicate that MTX may be a feasible alternative to daily cyclophosphamide in patients whose illness is not immediately life-threatening or in whom prior cyclophosphamide therapy produced significant toxicity. A phase I/II trial of high dose intravenous immunoglobulin in the treatment of systemic vasculitis has been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00358-11 LIR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenesis of *Chlamydia trachomatis* Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins University, (C. Gaydos, L. Bobo, R. Viscidi, S. West, J. Zenilman)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIH, NIAID, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the US with an estimated 5 million cases annually. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop improved diagnostic assays and to examine the pathogenesis of chlamydial infection. Studies of genital chlamydial infection in patients attending sexually transmitted diseases clinics have demonstrated a prevalence of 15% in men and women with and without symptoms. Prevalence varied little by either sex, symptoms, number of sex partners or the patient's relationship to his sex partners, although co-infection with *N. gonorrhoeae* and *C. trachomatis* was quite frequent in this population. Utilization of polymerase chain reaction (PCR) nearly doubled the sensitivity of culture for the detection of *C. trachomatis*. Among sexual partnerships infection was identified by PCR in 75% of male partners of infected females compared to only 39% by culture. Sequence analysis of amplified chlamydia DNA from partners identified the same serovar and further supported the accuracy of this assay. In an evaluation of a new molecular assay called ligase chain reaction (LCR) we identified chlamydia infection in 17.8% of urine samples collected from 1,500 symptomatic and asymptomatic men compared to only 9% by urethral culture. Screening of urine samples with molecular amplification assays may provide a highly cost-effective, noninvasive method for widespread screening of chlamydia. We also developed a new serologic technique for the measurement of specific antibodies to major outer membrane protein MOMP by producing a radiolabeled protein following PCR amplification, DNA transcription, and translation of MOMP protein. This serologic assay was then utilized to demonstrate a serologic rise in specific anti-MOMP antibodies in MOMP-vaccinated primates, which directly correlated with decreased infectious load. Clinical studies utilizing primer sets from the 16s rRNA of *C. pneumoniae* have demonstrated a marked increase in sensitivity and specificity of PCR for detection of *C. pneumoniae* in clinical pneumonia. Utilizing this assay, *C. pneumoniae* was identified in approximately 8% of nearly 700 patients with respiratory disease and in none of asymptomatic individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00537-05 LIR

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenic Mechanisms of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	G. Poli	Visiting Scientist	LIR, NIAID
	G. Pantaleo	Visiting Scientist	LIR, NIAID
	S. Stanley	Medical Officer	LIR, NIAID
	P. Bressler	Senior Staff Fellow	LIR, NIAID
	C. Graziosi	Visiting Associate	LIR, NIAID
	P. Biswas	Guest Researcher	LIR, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Terminated 1992 - 1993

LABORATORY OF MOLECULAR MICROBIOLOGY
1993 Annual Report

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LABORATORY OF MOLECULAR MICROBIOLOGY

SUMMARY

The Laboratory of Molecular Microbiology (LMM) applies molecular and biological techniques to study the structure, function, and regulation of prokaryotic and eukaryotic genes. Programmatically, murine retroviruses, the human immunodeficiency virus (HIV), human T cell leukemia virus type 1 and mycoplasmas and their host cells are investigated. Members of the LMM are physically located in Buildings 4 and 10 on the Bethesda campus and in Building 550 at the Frederick Cancer Research Facility. Although the research activities conducted by the LMM staff are quite diverse, a common theme involves the characterization of genetic elements (both chromosomal and episomal) affecting the interaction of microorganisms and the cells they infect.

STUDIES OF THE HUMAN IMMUNODEFICIENCY VIRUS

Molecular biological and virological techniques have been employed to evaluate structure/function relationships of structural and regulatory gene products encoded by the human immunodeficiency virus (HIV) during acute and chronic virus infections. In contrast to most other groups investigating HIV, LMM scientists have tended to focus on the effects of mutated viral genes in the context of the *complete* viral genome; complementary *in vitro* analyses of isolated HIV genes have also been carried out.

Quantitation of HIV-1 infection kinetics. The critical parameter affecting the kinetics of HIV-1 infection is the infection rate constant $k = \ln n/t_1$, where n is the number of infectious virions produced by one cell (100 to 300 for most HIV-1 isolates examined to date) and t_i is the time required for one complete cycle for virus infection (usually 3 to 4 days). This analytic tool was used to demonstrate that a TAT minus mutant of HIV-1, which exhibited a delayed replication phenotype (peak RT production at 5 weeks post infection), produced 12 rather than the wild type 180 infectious units per infectious cycle. The derived equations are also being exploited to ascertain neutralization titers directed against freshly isolated virus which are unable to induce syncytia formation in standard neutralization assays.
(Dimitrov and Martin)

Mutagenesis of the U5 region of the HIV LTR. Deletion mutations were introduced into the 5′, middle, and 3′ thirds (approximately 25 to 28 bps) of the HIV LTR U5 component of an infectious molecular proviral DNA clone and virus stocks were generated following transfection/cocultivation. Deletion of the middle third had little if any effect on HIV infectivity whereas elimination of the 5′ and 3′ thirds markedly compromised replicative capacity. The 5′ deletion caused a 10 to 20-fold reduction in the amount of genomic RNA packaged into progeny virions, a result implying the existence of a packing signal in this region of the U5. In contrast, analysis of virus containing a deleted 3′ U5 revealed normal amounts of viral RNA and structural proteins. However, high multiplicity infections of CD4 positive T cell lines indicated marked impairment of unintegrated viral DNA synthesis and suggested that the 3′ mutation compromised the reverse transcription reaction.
(Vicenzi and Martin)

Characterization of a "second site" revertant following the propagation of a viral mutant containing a partially deleted U5 region of the HIV LTR. A deletion mutation (26 bps) affecting the 3′ third of U5 was introduced in the 5′ LTR of the infectious molecular clone, pNL4-3. When the progeny virus, resulting from the transfection of HeLa cells, was used to infect MT4 cells at

low multiplicity, no evidence of replication competent virus was detected. However, in 1 of 4 separate high input multiplicity infections, virus replication was observed 30 days post infection. This virus progeny was then used to reinfect MT4 cells and exhibited infection kinetics indistinguishable from wild type. Direct PCR sequencing of the proviral DNA from these infections revealed that the reverted provirus had extended the original mutation an additional 19 bps. In the context of an HIV mutant defective in reverse transcription, the location of the revertant change could potentially alter the structure of the 5′ terminus of the RNA template and possibly restore a defect occurring at an early step of viral DNA synthesis.
(Vicenzi, Migone, Martin)

Construction of a Tat-minus mutant of HIV-1. HIVs were generated that replicate in the absence of the viral transactivator Tat as a result of substituting the CMV immedicate early enhancer segment for the HIV-1 NF-kB/Sp1 or Sp1 sites in the viral LTR. Three groups of *tat* deficient viral mutants were studied: Group 1 mutants contained a single stop codon and a missense mutation near the N-terminus of the tat ORF; Group 2 mutants contained the same tat mutation as in group 1, plus two additional stop codons further downstream; Group 3 mutants bore changes in the first six codons of tat ORF plus a 44-nt deletion and a 22-nt polylinker insertion.

Following transfection into HeLa cells, all of the *tat* mutants produced detectable amounts of viral mRNAs and released reverse transcriptase activity into the medium. When these *tat*-minus viruses were studied in tissue culture, only group 1 and 2 mutants (containing CMV-LTRs) were infectious but exhibited markedly delayed replication kinetics (2 to 3 weeks slower than WT). The group 1 and 2 *tat* mutants containing CMV-LTRs produced large amounts of progeny virions at time of peak RT. None of the *tat*-minus HIVs containing wt LTRs generated detectable virus progeny. Extensive sequence analyses and functional assays verified that these viruses were indeed *tat*-defective. Interestingly, one of the group 3 *tat* mutants established a chronic infection in MT4 cells which became a high producer of progeny virions but exhibited no HIV-induced cytopathicity. RT-PCR and sequencing of the particles released from these chronically infected cells indicated that both the CMV enhancer and the *tat* mutation were present and unaltered. RNase mapping of viral RNA indicated that the transcriptional start site of the mutant was indistinguishable from the wild type HIV-1. The tat-minus viruses and the chronically infected cells are being further characterized.
(Chang, Martin)

The contribution of TATA box changes to HIV-1 LTR-directed expression. Two types of second-site revertant changes involving the TATA motifs appeared in an ordered fashion following long-term passage of HIV containing mutations (deletion or point changes) affecting the three Sp1-sites. When introduced into the original LTR mutant proviral clones, the TATA revertant changes markedly accelerated virus replication. In complementary *in vitro* studies, the TATA motif base substitutions increased the rate of TFIID/TFIIA assembly and stability on the HIV template. The TATA box revertant changes also increased basal levels of transcription five to ten fold in the context of the wild type LTR when assayed in an *in vitro* transcription system.
(Shibata and Martin)

Characterization of inhibitory elements within HIV-1 genomic RNA. Two independent regions within the HIV-1 gag/pol gene were identified which inhibit expression of HIV mRNAs. The effect of these inhibitory regions (IRs) was demonstrated in two reporter gene constructs and was found to be dependent upon the orientation of the mRNA sequences. IR-containing mRNAs were detected as spliced and unspliced transcripts in the nucleus, but not in the cytoplasm of transfected cells indicating that expression was blocked post-transcriptionally. The inhibitory effect of IR sequences was observed in several primate cell lines, but was not detected in mouse 3T3 cells. To analyze the nature of this inhibition, we first determined whether the inhibitory effect of gag/pol sequences could be reversed by the rev gene product in *trans*, and the Rev-responsive element (RRE) in *cis*. In HeLa cells, Rev rescued the expression of CAT reporter plasmids containing both the IR and RRE sequences; no effect of Rev was detected on the expression of CAT gene containing only the IR or RRE sequences alone. Thus, in human cells, the effect of Rev required the presence of IR sequences. In contrast to the inhibitory effect detected in human cells, IR sequences did not inhibit CAT gene expression in mouse 3T3 cells, demonstrating that the IR effect was cell-type dependent and suggesting that cellular factors may be required for the inhibition. Studies are in progress to clarify the nature of the inhibitory effect of *gag/pol* sequences in human cells and to identify cellular factors which interact with these regions.

(Berthold, Maldarelli, Martin)

Alternative mRNA splicing of HIV modulates viral replication and infectivity. Alternative splicing of RNA is a mechanism that is used to regulate gene expression during cell growth and differentiation. The role of this mechanism has been examined in regulating the replicative capacity of HIV. Many of the splice donor and acceptor motifs in the infectious clone of HIV, pNL4-3, were mutated yielding viruses that fail to produce the full complement of spliced viral mRNAs. Using a semi-quantitative PCR assay to determine the relative proportions of the alternatively spliced HIV mRNA species, these HIV mutants and several macrophage-tropic isolates were shown to direct altered patterns of RNA splicing. Analyses of the biological and biochemical properties of these viruses indicated that the relative proportions of the different mRNAs for the envelope, Nef and Rev proteins, determines the efficiency of their expression and thereby affects the replication rate and infectivity of these viruses. The expression of alternatively spliced HIV mRNAs in different cell types from HIV infected patients at different stages of disease is currently being examined using the PCR assay to ascertain the correlation between RNA splicing differences and progression of HIV infection to AIDS.

(Purcell, Englund, Martin)

Tat functions as an adaptor between enhancers and TATA binding factors. The spatial relationship among the Spl, TATAA, and Tat motifs in the HIV-1 promoter has been examined for its effect on Tat-induced trans-activation of HIV LTR-directed expression. There was no significant fluctuation of the basal activities of the HIV-1 promoter when the distance between Spl and TATAA was increased. In fact, progressively higher basal activities were observed when the Spl-TATAA distances increased from 26 bp up to 65 bp. However, increasing the Spl-TATAA spacing did have a profoundly detrimental effect on Tat-mediated trans-activation. Our results suggest that Spl, TATAA-binding factors, and the Tat protein must contact each other for optimal Tat function. We propose that Tat acts as an adaptor between Spl (or other upstream enhancers) and TATAA-binding factor(s) in forming a multi-protein complex which enhances transcriptional activity from the HIV-1 LTR.

(Huang and Jeang)

Role of Tat and cellular factors in HIV expression. Viral and cellular factors influence HIV-1 expression. Tat, TAR RNA, and host cell proteins are all important components in this process. One of our goals is the isolation and characterization of important cellular factors. Using a binding affinity assay for RNA-binding proteins, a cDNA was isolated that encodes the TAR RNA binding protein, TRBP. We have defined the RNA binding domain of this protein which allowed us to conclude that it belongs to a new family of double-stranded RNA-binding proteins that includes dsI kinase and drosophila Staufen. By DNA, RNA, and protein analyses, we detected TRBP in a variety of mammalian cells. Using immunoprecipitation and nuclease Sl protection of RNA, it could be shown that TRBP and TAR RNA are complexed during HIV infection. This result suggests a functional role for TRBP during virus production.

(Gatignol and Jeang)

Cooperation of RNA binding and protein oligomerization activities of Rev are required for *trans*-activation of RRE RNA: It has been previously demonstrated that direct interaction of Rev with the Rev responsive element (RRE) RNA is not an absolute requirement for trans-activation. Rev activated a heterologous RNA if directed to it by another RNA binding protein [e.g. by the MS2 phage operator sequence (RREZ-MS)]. Although the binding of MS2 RNA was directed by the coat protein moiety, changes at the RNA binding and nucleolar localization (NLS) domain(s) of Rev (residues 35-50) in the context of the Rev/MS-C fusion protein abolished the Rev response for both the RRE and the MS2 RNAs. To examine the nature of the cooperative interactions between the RNA binding and protein oligomerization domains of Rev, the RNA binding motif of Rev was replaced with a poly-arginine tract both in the context of Rev and the Rev/MS-C fusion protein. Functionally defective of Rev/MS-C fusion protein mutants, which had lost the presumptive RRE RNA binding motif (residues 35-50), regained the activation potential for both RRE and MS2 RNA when 9 ARGs were inserted at the deletion. Rev response was not restored with fewer than 8, or more than 11 ARGs. Poly-arginine insertions in place of a deletion that removed residues 24-60 of Rev preserved the activation for MS2 RNA, but not for RRE. Poly-arginine insertions that preserved the Rev responsiveness localized to the nucleoli; those with poor or no Rev response accumulated mostly in the cytoplasm. From these genetic studies, we propose that a poly-arginine insertion near the N-terminus of Rev promotes nucleolar targeting in a context sensitive manner, and the Rev sequence immediately flanking the 9 arginines (residues 24-35, and residues 36-50) is required for RRE RNA binding. The N-terminal region between residues 24 and 35, and between 50 and 55 are involved in protein oligomerization and appear to be essential for activation of RRE RNA.

(Nam, Gerstberger, and Venkatesan)

A cellular protein that binds HIV RRE RNA and several other structured RNAs is an inhibitor of the interferon induced PKR kinase in vitro and in vivo: We have cloned and characterized a cellular protein, RBF that binds to the HIV-1 Rev responsive element (RRE) RNA. RBF was identical to the previously reported HIV-1 TAR RNA binding protein (TRBP). *E. coli* expressed RBF/TRBP displayed binding affinities for a broad range of RNAs, with a 11 bp GC rich RNA duplex constituting the minimal binding target. RBF/TRBP contains two basic motifs with significant homology to many viral and cellular RNA binding proteins including the interferon inducible double stranded (ds) RNA activated PKR (DSI or DAI) kinase and the vaccinia virus encoded E3L protein that is a PKR kinase inhibitor. In vitro, RBF/TRBP was a potent competitive inhibitor of ds RNA activation of PKR kinase. RBF/TRBP also inhibited PKR kinase and eIF-2a phosphorylation in vivo in a transient expression system in which translation of a reporter gene was inhibited by localized activation of PKR kinase. Under transient expression conditions in HeLa cells, RBF/TRBP complemented the E3L mutant of vaccinia virus that was defective for virus replication and protein synthesis. RBF/TRBP may thus represent cellular regulatory protein(s) that bind RNAs containing specific secondary structure(s) and inhibit the PKR kinase in a localized manner.

(Park, Nam, and Venkatesan).

Mutational analysis of the matrix protein of HIV-1. A large number of single amino acid changes have been introduced into HIV-1 matrix (MA). The effects of these mutations on virus replication on T cells and primary macrophages, virus particle assembly and release, Gag processing, and envelope incorporation into virus particles are being analyzed and have revealed sequences involved In HIV-1 particle assembly and release. These investigations have also shown that a portion of MA which bears sequence homology to picornavirus Vp1 and which has been postulated to play a role in HIV-1 entry is not critical to infection of T cells or primary macrophages. Efforts are currently being directed towards further defining domains and specific amino acid residues involved in MA function.

(Freed, Martin)

Integration of HIV-1 in Primary Monocyte-Derived Macrophage. The literature involving the requirement for integration of lentiviral DNAs during productive infection is replete with ambiguity and conflicting reports. Using an M-CSF dependent macrophage culture system developed in our laboratory and a full length infectious molecular clone corresponding to the HIV-1 macrophage-tropic isolate, AD-87, we have investigated the requirement for integration during productive infection of monocyte derived human macrophages (MDMs). An in-frame deletion of the D(35)E affecting the DNA binding domain of the HIV integrase was introduced into the cloned AD-87 provirus and virus stocks were generated by transfecting HeLa cells. The mutation ablated virus infectivity in both PBMCs and MDMs. A PCR-based kinetic analysis of viral DNA production demonstrated the production of unintegrated viral DNA in both cell types but subsequent steps in the infectious cycle were blocked. In contrast to wild type virus infected cells where levels of newly synthesized DNA rose and over the initial 96 hrs of infection, the newly synthesized DNA directed by the integrase deficient virus peaked at 24 h and diminished markedly thereafter. These results demonstrate that integration is required for HIV-1 infection of MDMs.

(Englund and Martin)

Mechanism of adaptation to growth in tissue culture cell lines. Virus derived from the infectious molecular clone pELI1 was adapted by passage to grow in the H9 T cell line, and the passaged virus had an enhanced replicative capacity and broadened host range. Mutations in the CD4 binding region of gp120 and in the fusogenic region of gp41 were found to account for the altered phenotype. The envelopes of the virions of these variants were investigated for structural alterations in an effort to correlate the changed biological properties of the viruses with changes in the biochemical properties of their envelopes. The Met-7 to Val change in the fusogenic region of gp41, which allowed ELI to replicate in T cell lines, had little effect on the spontaneous shedding of gp120 from virions but increased both the CD4-induced release of gp120 and increased the affinity of virions for the soluble CD4 derivative CD4-IgG. Acquisition of the Gly-427 to Arg mutation in gp120 in the background of the Val-7 gp41 change, which conferred the ability to replicate in the promonocytic cell line, U937, increased both the spontaneous and CD4-induced shedding of gp120 as well as further increasing the affinity of the virions for CD4-IgG. Thus, with this virus, there is a strict correlation with the capacity to replicate in cell lines and both the CD4-induced dissociation potential of gp120 from virions and the affinity of virions for CD4-IgG. These results demonstrate that the CD4-binding activity of gp120 in virions and the ability of gp120 to dissociate from virions can be influenced not only by changes in the gp120 component but also by the particular gp41 component with which it is coupled.

(Willey, Martin and Peden)

Structure and Function of the HIV-1 Envelope Glycoproteins. The HIV-1 envelope glycoproteins, gp120 and gp41, mediate viral attachment and penetration into CD4+ cells; some isolates of HIV-1, however, are restricted in their ability to productively infect primary macrophages or continuous CD4+ cell lines. In order to study the biochemical basis for these differences, a variety of infectious molecular clones of HIV-1 containing defined mutations within the envelope glycoproteins were analyzed employing radiolabeled purified virions to evaluate envelope structure and function. The tendency of purified virions to release their gp120 envelope component either spontaneously or after interacting with a soluble form of CD4 increased in parallel with the ability of different HIV-1 variants to grow in CD4+ cell lines. In contrast, both spontaneous and soluble CD4-induced release of gp120 from virions of macrophage-tropic HIV-1 variants was substantially reduced. Work is currently in progress to determine the relationship between these altered physical and functional properties and the ability of HIV-1 to productively infect different cell types.

(Willey and Martin)

Analysis of HIV-1 envelope variable region interactions. A number of chimeras between the *env* genes of the NL4-3 and WMJ-2 proviral clones have been constructed and analyzed for function. The exchange of the first and second variable domains (V1/V2) of gp120 leads to a loss in syncytium forming ability and a partial block in gp160 processing. Virus bearing the exchanged envelope is also defective in the infection process; however, long-term passage of infected cultures resulted in the emergence of a reverted virus population. Characterization of env sequences from cells infected with reverted virus indicates that a single amino acid change in the major CD4 binding domain is sufficient to confer the revertant phenotype. The effects of this single amino acid change on CD4 binding and on replication of wild-type virus is being evaluated.

(Freed, Martin)

Characterization of envelope interactions involved in infection of primary human macrophages. Recombinants have been constructed between the *env* genes of the AD8 and SF162 macrophage-tropic HIV-1 isolates. Recombinants in which a region of envelope extending from V3 to gp41 has been exchanged are defective for growth in cultures of primary human macrophages. This defect appears to be macrophage-specific, as all recombinants replicate with comparable efficiency in PBMC. Results obtained from these recombinants also suggest that virus bearing the SF162 envelope is very dependent on the presence of Vpu while virus bearing the AD8 envelope are relatively vpu-independent. Additional recombinants are being constructed to further define the envelope interactions required for efficient macrophage infection and to further characterize the isolate-specific requirement for Vpu.

(Freed, Martin)

Expression of HIV envelope in permanent cell lines. A retroviral vector containing the neomycin antibiotic resistance gene and the HIV *rev* and *env* genes under control of a CMV promoter was constructed. Transfection of an amphotropic packaging cell line resulted in the production of infectious virus which conferred neomycin resistance and stable expression of HIV envelope. Long-term expression of HIV envelope has previously been difficult to achieve. The engineered retroviral construct will be used in experiments designed to generate resistance to HIV, analogous to the Fv-4 model of retroviral resistance in mice (described above).

(Fujita, Silver)

Structural and functional analysis of the HIV-1 Vpu gene product HIV is a complex retrovirus containing a number of genes not commonly found in other retroviruses. One of these genes, Vpu, which is encoded only by HIV-1 and does not have any known homologues in HIV-2 or SIV, encodes a small integral membrane protein. In the past we have identified two biological activities for Vpu: (i) enhancement of particle release; (ii) degradation of CD4. To study the effect of Vpu on particle release, a series of mutations was introduced into the Vpu gene and/or the Env gene of the infectious molecular clone, pNL43. Mutant viral constructs were analyzed in transient expression assays in HeLa cells by pulse/chase analyses for their ability to produce and release virus particles. These studies demonstrated that the production of Env is not required for HIV-1 particles to be secreted into the culture medium. Particle release was Vpu dependent in the presence or absence of Env indicating that Vpu enhances particle release through a mechanism that does not involve the Env product.

(Willey, Maldarelli, Strebel)

Mechanism of CD4 degradation by Vpu: *in vitro* model. To study the mechanism of Vpu-induced CD4 degradation, an *in vitro* assay was developed which involves the production of both Vpu and CD4 in rabbit reticulocyte lysates containing canine microsomal membranes. This simple *in vitro* system can be used to assess parameters (including potential inhibitors) of Vpu activity. A series of *in vitro* expression plasmids was constructed that directed the synthesis of wild-type CD4 or Vpu or specific mutants of both proteins *in vitro*. As is the case in tissue culture systems, Vpu induced degradation of CD4 *in vitro*. Successful CD4 decay required the presence of Vpu and CD4 in the same membrane compartment. Co-expression of CD4 and Vpu in rabbit reticulocyte lysate in the absence of microsomal membranes did not result in any appreciable destabilization of CD4. Analyses involving specific mutants of CD4 and Vpu indicated the (i) glycosylation of CD4 is not a prerequisite for degradation; (ii) CD4 decay is post-translational; (iii) Vpu targets a short sequence in the cytoplasmic tail of CD4; (iv) degradation *in vitro* is significantly slower than *in vivo*, yet there are no degradation intermediates detectable; therefore, (v) CD4 decay is likely to be a multi-step process; (vi) short deletions from the C-terminus of Vpu or an internal highly conserved domain result in a loss of activity.

(Chen, Strebel)

Cellular localization of the HIV-1 Vpu protein. The product of the HIV-1 *vpu* gene is an 81 amino acid amphipathic membrane bound phosphoprotein. Vpu is an integral membrane protein with the hydrophilic C-terminal domain of Vpu projecting into the cytoplasm (a type 1 integral membrane orientation). Vpu has two distinct effects on HIV-1 replication: it increases the efficiency of virion release and decreases the half-life of the HIV-1 receptor protein, CD4. It is not known whether Vpu affects virion production and CD4 degradation directly (by Vpu-protein interactions) or indirectly (by altering membrane function) perhaps as an effect on ion channel activity. In order to understand the mechanism of Vpu action on these two distinct biochemical events, we have examined the intracellular location of the Vpu protein. Employing cell fractionation techniques, we have demonstrated that Vpu is synthesized on heavy membranes of the rough endoplasmic reticulum and subsequently trafficks into a light membrane compartment. These data suggest Vpu may affect such events as virion production and CD4 degradation as it trafficks through different cellular compartments. Immunofluorescence studies, using a polyclonal rabbit anti-vpu antisera, complimented these studies, and indicated that Vpu was concentrated in perinuclear regions and in vesicles distributed throughout the cytoplasm. Immunofluorescence co-localization studies have not revealed an association of Vpu with Golgi, endosomal, or endoplasmic reticulum markers. Further co-localization studies are in progress to define precisely the intracellular Vpu compartment. Isolated membrane fractions containing Vpu are also being used to determine whether Vpu affects membrane transport functions.

(Maldarelli, Martin, Strebel)

Characterization of Nef mutants of HIV-1 and HIV-2. Mutations were constructed in the *nef* genes of several strains of HIV-1 and HIV-2. Nef mutants of the LAI, MAL, and ELI strains of HIV-1 and the ROD strain of HIV-2 consistently produced less virus and had slightly delayed replication kinetics in peripheral blood mononuclear cells (PBMC) compared with the corresponding wild type viruses. The effect of Nef on virus replication was more variable when tested on various CD4-positive cell lines of either the lymphocytic or monocytic lineage. In some cases, there was no detectable difference in the replication kinetics between the Nef mutant and wild type viruses. For example, mutation of the *nef* gene of the LAI strain of HIV-1 and the ROD14 strain of HIV-2 had little effect on virus replication in CEM cells and Jurkat cells, respectively. However, in SupT1 cells, Nef mutants of HIV-1_{LAI}, HIV-1_{MAL}, and HIV-2_{ROD14} were found to have a delayed time course of infection, as were Nef mutants of HIV-1_{LAI}, HIV-1_{ELI1}, and HIV-1_{ELI6} in Jurkat cells, and Nef mutants of HIV-1_{ELI5} and HIV-2_{ROD14} in CEM cells. In promonocytic U937 cells, no effect of Nef could be detected. These results demonstrate that the effects of Nef are variable and depend on the particular host-virus system, and that Nef is a positive factor for the replication of HIV *in vitro*.

(Ryan and Peden)

HIV-1 Nef protein modulates the cell surface expression of the CD4 receptor: The functions of the HIV-1 Nef protein in the life cycle of the virus have remained elusive. We are examining the cellular physiological roles of Nef, notably, the down-regulation of CD4 receptor and other cytokines. The number of cells expressing surface CD4 was reduced approximately 10-fold in samples cotransfected with a Nef expression plasmid as compared to the controls. At low levels of Nef expression, the effect on cell surface expression was profound; increasing amounts of Nef resulted in a dose-dependent reduction in the steady-state levels of CD4 that approached 90%. Nef expression did not modulate CD8, an ER retention CD4 mutant, CD4-KDEL, or a hybrid containing the extracellular domain of CD8 and the intracellular (cytoplasmic) domain of CD4. A CD4 mutant that excised the cytoplasmic tail of the protein was, however, susceptible to Nef effect. These studies suggested that the cytoplasmic domain of CD4 may not be target for Nef mediated down-regulation. Mutations that forced Nef initiation from the internal MET codon at position 20, and a mutation that abolished myristoylation by replacing the GLY with ALA at positions 2 and 3 severely impaired the Nef effect on CD4. A mutation that exchanged the THR at position 15 (presumptive PKC phosphorylation site) for ALA preserved the CD4 effect, while mutating the internal THR residues at 44 and 48 reduced in the magnitude of CD4 down-regulation.

(Hiller and Venkatesan).

Role of the *nef* gene during HIV-1 replication in tissue cultures. Highly conserved in primate lentiviruses, the Nef protein is essential for the sustained replication and production of "high virus loads" in the infected host, and for AIDS pathogenesis. The role of Nef on HIV replication is tissue culture systems has been difficult to ascertain. The replicative capacities of Nef defective virus were tested on CD4 positive T cell lines and peripheral blood mononuclear cells (PBMCs). The kinetics of Nef defective viral replication in Jurkat cells (lymphocytic origin) and U937 (monocytic origin) were indistinguishable from wild type virus. When PBMCs, prestimulated with phytohemagglutinin-P, were infected with Nef defective virus, infectivity was diminished 5 to 7 fold. Moreover, the mutant virus was severely impaired in its ability to replicate following mitogen induction of previously infected resting PBMCs. These data suggest that the nef gene does not affect viral replication in T cell lines but is a positive factor for HIV replication in primary cells.

(Vicenzi and Martin)

Ribozymes targeted to the HIV-1 genome. Ribozymes are small RNA molecules capable of cleaving an RNA substrate. The specificity of digestion is determined by the presence of a GUC motif in the target RNA indicating that many HIV sequences are potential ribozyme cleavage sites. We have targeted ribozymes to five sites. Ribozymes directed to the U5 and R regions of the HIV-LTR are most efficient, while ribozymes that target the polymerase, envelope and RRE regions are less so indicating that some sites on the HIV-1 RNA may be better substrates than others. We have also examined the ability of ribozymes to inhibit gene expression in transgenic disease models. Mice that contain an HIV-1 provirus may exhibit characteristic pathologies including lymphadenopathy, skin papillomas and proteinuria. When such mice were crossed with transgenic mice expressing an U5 ribozyme, an inhibition of HIV RNA expression and a delay in the onset of proteinuria was observed. This indicates that (1) ribozymes can inhibit gene expression in transgenic mice and (2) that ribozymes can reverse some of the pathological manifestations of an integrated HIV genome. Furthermore, because ribozyme-containing mice are themselves disease free this implies that ribozyme expression, *per se*, presents no toxicity to the intact animal.

(Dropulic, Dickie, Jeang)

Construction of an infectious molecular clone of a macrophage-tropic HIV-1 isolate. A full length infectious molecular clone of the macrophage tropic virus strain, HIV_{AD-87} was isolated from infected human PBL DNA as a permuted circular proviral DNA containing a single LTR. The virus produced following transfection of Hela cells replicated in human macrophages and PBLs but not in continuous T-cell lines. To facilitate these studies, the AD-87 molecular clone was converted to a two LTR proviral DNA molecular clone. A complete nucleotide sequence revealed that the *vpu* gene was disrupted; site-specific mutagenesis was then used to convert this clone to vpu positive status. Transient transfections in Hela cells indicated that the *vpu* positive clone directed production of increased progeny virion and envelope proteins. It is anticipated that the HIV_{AD-87} molecular clone will be used to assess the effects of LTR, *env*, *vif*, and *vpr* gene mutations during infections of human monocyte derived macrophages.

(Theodore, Englund, Martin)

Identification of HIV-1 isolates capable of infecting chimpanzee PBMCs. More than 90% of chimpanzees inoculated with HIV-1 have received derivatives of the $HIV\text{-}1_{LAI/IIIB}$ isolate. These inoculated chimps invariably become infected, produce antibodies to viral proteins, but fail to develop disease. In an attempt to develop a disease model of AIDS in HIV-1 infected chimpanzees, HIV-1 isolates were screened from seropositive, symptomatic individuals for their ability to replicate in chimpanzee peripheral PBMCs. Of 23 samples freshly isolated from AIDS or ARC patients, 3 isolates (DH012, 20, and 29) could readily infected chimp PBMCs *in vitro*. DH012 and DH029 also readily infected human monocyte derived macrophages. DH012 replicated more rapidly and to higher titers in chimpanzee PBMCs compared to a "chimp-adapted" derivative of HIV_{IIIB}. HIV-1 $_{DH012}$ also induced marked CPE in both human and chimpanzee PBMCs and was able to infect PBMCs from 16 different animals including a sample from a chimpanzee that had been previously inoculated and continued to be persistently infected with $HIV\text{-}1_{IIIB}$. The nucleotide sequence of the V3 envelope regions of the three "chimp-tropic" HIV-1 isolates were unremarkable. $HIV\text{-}1_{DH012}$ (alone) and a mixture of the three chimp-tropic HIV-1 isolates will be used to infect chimpanzees.

(Shibata and Martin)

Transgenic mouse model of HIV-1 induced pathogenesis. The 5′ LTR of an HIV-1 infectious molecular proviral DNA clone was modified to increase expression in mouse cells by replacing sequences of the NFk-B enhancer with transcriptional regulatory elements from Moloney murine leukemia virus. Two lines of transgenic mice have been established that contain the modified genome and, as assayed by Northern analysis, express HIV RNA in several tissues, most notably in spleen and muscle. Co-culture of transgenic spleen cells with human peripheral blood lymphocytes results in the production of infectious virus. Both lines of heterozygous transgenic mice exhibit a "wasting" disease and increased susceptibility to opportunistic pathogens, which is not seen in non-transgenic sibling cagemates. Around two months of age, the mice develop eye abcesses from which *Pasteurella pneumotropica* has been isolated. Hair loss, associated with infection by three different species of mites, has also been observed in many animals. Most striking is the progressive weight loss with an absence of body fat and loss of muscle mass despite normal food intake. Animals become moribund at approximately six months of age. No gross pathology is apparent at necropsy and histological examination of tissue has revealed necrosis of muscle fibers without evidence of inflammation. Cells of the immune system are being characterized and assayed for functional capabilities with the aim of developing a murine model that can be used to investigate the molecular basis for HIV induced pathogenesis and to test the efficacy of potential therapeutic agents.

(Dickie, Mounts, Chang, Martin)

Pathophysiology of HIV-1 Nef gene expression in transgenic mice. In HIV transgenic mouse lines, HIV Nef gene expression has resulted in the development of cataracts and proliferative skin lesions resembling papillomas. Epidermal proliferation was induced when animals were exposed to UV light, a treatment known to activate the HIV LTR. Multiple lines expressing Nef from transgenes constructed by fusing the HIV LTR to the Nef gene alone have also exhibited a very high incidence of fetal death. Fetal death was not observed in transgenic mouse lines bearing HIV proviral constructs even though these animals also expressed high levels of Nef protein. This apparent contradiction may reflect temporal or tissue specific differences in the regulation of Nef or the possible interaction of Nef and one or more of the HIV encoded proteins. In this regard, mating Nef mice with HIV provirus mice lacking a functional Nef gene has increased the incidence of fetal death above levels typical for the Nef mice alone. *In utero* expression of Nef is being examined as a cause of developmental arrest.

(Dickie and Martin)

Sensitive assay for reverse transcriptase. An extremely sensitive assay for reverse transcriptase (RT) based on RT-PCR has been devised. Samples suspected of containing RT activity are tested for their ability to convert a Brome Mosaic Virus RNA template to PCR-amplifiable DNA. This assay is 100,000 times more sensitive than conventional assays for RT activity. Perhaps because of its extreme sensitivity, the new assay detects RT activity in extracts from normal cells. The assay is being modified to make it more specific for particular retroviruses (such as HIV) and capable of distinguishing exogenous retroviruses from the RT activity present in normal cells.

(Fujita, Repaske, Maudru, Silver)

OTHER RETROVIRUSES

Replication-competent retroviruses arising during gene therapy in rhesus macaques. During a safety trial of a retrovirus-based vector for gene therapy conducted in a group of rhesus macaques by scientists in NHLBI, contaminating replication-competent recombinant murine retroviruses present in a "control" inoculum induced rapidly progressing T-cell lymphomas in three of five infected animals; the biologically active retroviruses present in tumor tissue and blood plasma was characterized. RNA extracted from the tumor was used in RT-PCR experiments that would amplify any spliced env mRNA related to the murine C-type retroviruses, the source of the vector. Two general types of env RNA were characterized: the first contained a typical amphotropic MuLV envelope structure and the second harbored a recombinant MCF-like derivative of the original amphotropic envelope. Virus containing the amphotropic envelope was present in all affected animals including a previously unreported viral recombinant containing an LTR found only in Moloney murine leukiemia virus. Infection of primary rhesus fibroblasts, but not peripheral blood mononuclear cells, was readily established. The novel recombinant Moloney (LTR)-amphotropic virus grew to significantly higher levels than prototypic amphotropic virus in rhesus fibroblasts.

(Purcell and Martin)

Mouse model of retroviral resistance due to expression of retroviral envelope gene. Transgenic mouse strains expressing high levels of the *Fv-4* retroviral envelope gene were completely resistant to infection with Friend murine leukemia virus (no virus could be detected in blood or spleen from 5 days to several weeks following infection). Transgenic strains that expressed lower amounts of the transgene were partially resistant (*i.e.* they were infectible but recovered from viremia after several weeks). Recovery from viremia suggested that an immune response is involved in this resistance. Complete resistance was transferred to control mice via bone marrow transplantation from resistant donors. Partial resistance (ability to recover from viremia) was conferred by transplantation of a 1:1 mixture of bone marrow from resistant and susceptible donors. We have made a retroviral vector system capable of expressing the *Fv-4* envelope gene in order to see if resistance can be induced by gene therapy. This model system has implications for possible gene therapeutic approaches to the treatment of AIDS.

(Limjoco, Silver)

Identification of mouse mammary tumor viruses involved in the Mlsf system. The products of minor lymphocyte stimulating (Mls) genes are responsible for the clonal deletion of specific V-beta bearing T cells. Recent studies have shown that these Mls superantigens are encoded by MMTVs. We have analyzed a series of genetic crosses to identify endogenous Mtv loci responsible for the novel Mls determinant, Mlsf. We showed that at least 3 MMTVs, Mtv-8, Mtv-9, and Mtv-11 are involved in the expression of Mlsf, and that each of these 3 MMTVs exerts independent and distinct influences upon the subsets of T cells deleted by Mlsf.

(Kozak with Abe)

A novel tumor specific integration site for type B retrovirus induced lymphomas. Nonacute MuLVs can transform mouse cells following activation of proto-oncogenes by insertional mutagenesis. Our studies have described a new common viral integration region disrupted during tumor induction by an unusual retrovirus. This retrovirus, TBLV, shows over 98% homology with the mouse mammary tumor virus, but in contrast to MMTVs which induce mammary adenocarcinomas, TBLV induces T cell lymphomas. A novel proviral integration site was identified in 20% of the tumors examined. This site was mapped to a region on the X chromosome which is not known to contain protooncogene sequences.

(Kozak, with Ball).

Determination of functional domains of HTLV-I Tax. All transcriptional activators possess two properties: 1) targeting and 2) activation. These functions may not be separable into discrete domains. Using a GalTax fusion protein and an HTLV-I LTR promoter construct containing a Gal4 consensus binding site, we demonstrated that Tax has an activation domain separable from it targeting domain. To identify this region, TaxVP16 fusion proteins were made. The ability of functionally inactivating mutations in Tax to target the TaxVP16 fusions to the HTLV-I LTR was observed. The mutations mapped to amino acids 289-337.

(Semmes and Jeang)

CHROMOSOMAL MAPPING

Genetic linkage studies in the mouse. We have been involved in developing a multi-locus genetic map of the mouse using somatic cell hybrids and interspecies/intersubspecies genetic crosses. The progeny of these crosses have now been typed for over 450 loci on 20 linkage groups. Some of the genes mapped most recently include those encoding cyclin, microtubulin, heat shock proteins, and genes specifically expressed in nervous tissue. One gene, aggrecan (a major structural component of cartilage), mapped to a region of Chromosome 7 bearing the cmd (cartilage matrix deficiency) mutation. Sequence analysis identified a 7bp deletion in this gene in cmd mice indicating we have identified the defect responsible for this mutant phenotype.

(Kozak, Adamson)

MYCOPLASMAS

Mycoplasmas of human origin. In collaborative studies, *Mycoplasma fermentans* has for the first time been isolated directly from lung tissue at autopsy of two patients with fatal respiratory disease, and further isolations have confirmed the organism in the urine of AIDS patients. *Mycoplasma pneumoniae* has also been identified in fourteen cervical specimens with suggestions that the organism may play some role in genital tract disease. *Mycoplasma pirum*, heretofore of unknown origin, has been cultivated and identified in the lymphocytes of an AIDS patient. DNA amplification tests suggest additional occurrence of the organism in this locale.

(Tully)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00027-26

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joseph G. Tully	Head, Mycoplasma Section,	LMM,	NIAID
Others:	David L. Rose	Research Microbiologist,	LMM,	NIAID

COOPERATING UNITS *(if any)*

S-C. Lo, AFIP, Washington, DC;C. Bebear, Univ.Bordeaux II, France;R. Dular, Ontario Health,Ottawa,Canada;H. Ball,Ireland Vet.Services, Stormont.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Mycoplasma Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of Mycoplasma genitalium in human disease has been difficult to define. The organism is frequently mixed with M. pneumoniae and these two organisms share considerable immunologic reactivity. Thus, conventional complement-fixation tests or new immunosorbent assays for M. pneumonia infections can not differentiate serologic responses to each organism. We now have also identified mixed M. genitalium/M. pneumoniae strains in the synovial fluid of a patient with polyarthritis following primary pneumonia infection, suggesting that M. genitalium can become disseminated from either urogenital or oropharynx sites. We have currently defined an indirect immuno-fluorescence assay that will accurately distinguish colonies of each organism on agar plates when mixed strains are grown in culture. This test is being applied to specimens being compared in culture versus direct testing with a variety of molecular probes.

Collaborative studies have also established that animal mycoplasmas (M. arginini) can induce severe and fatal infections in immunocompromised patients. The inability of antibiotic drugs to control such infections is related to the mycoplasmastatic activity of known antibiotics and the contribution of an intact humoral and cell mediated defense in the host.

Taxonomic characterization of a new mycoplasma from the urine of both HIV-positive and AIDS patients has been carried out in collaborative studies. The high cytopathogenicity for a variety of eukaryotic cells appears related to the unique attachment structure on the organism and the ability of the organism to induce intra-cellular infections. The organism has been designated Mycoplasma penetrans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00190-15

PERIOD COVERED
October 1, 1993 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Molecular Genetics of Eukaryotic Cells and Their Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Martin	Chief	LMM, NIAID
	E. Vicenzi	Visiting Associate	LMM, NIAID
	Peter Dickie	Visiting Associate	LMM, NIAID
	Damian Purcell	Visiting Associate	LMM, NIAID
	Eric Freed	Staff Fellow	LMM, NIAID
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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multiple RNA splicing sites exist within human immunodeficiency virus type 1 (HIV-1) genomic RNA and these enable the synthesis of many mRNAs for each of several viral proteins. The effect of altered RNA processing was measured following mutagenesis of the major 5' splice donor and several cryptic, constitutive, and competing 3' splice acceptor motifs of the isolate, HIVNL4-3. Mutations that ablated constitutive splice sites led to the activation of new cryptic sites; some of these preserved function. Mutations that disrupted competing splice acceptor sites caused marked alterations in the pool of virus-derived mRNAs and, in some instances, markedly altered virus infectivity and/or the profile of virus proteins. The redundant RNA splicing signals present in the HIV-1 genome and alternatively spliced mRNAs provide a mechanism for regulating the relative proportions of HIV-1 proteins, and, in some cases, virus infectivity.

A number of chimeras between the *env* genes of the HIVNL4-3 and HIVWMJ-2 isolates have been constructed and analyzed for function. The exchange of the first and second variable domains (V1/V2) of gp120 leads to a loss in syncytium forming ability and a partial block in gp160 processing. Virus bearing the exchanged envelope is also defective in the infection process; however, long-term passage of infected cultures resulted in the emergence of a reverted virus population. Characterization of *env* sequences from cells infected with revertant indicated that a single amino acid change in the major CD4 binding domain is sufficient to confer the revertant phenotype. Chimeric viruses have also been constructed involving portions of the *env* genes of the HIVAD8 and HIVSF162, two macrophage-tropic HIV-1 isolates. Recombinants in which regions of the envelope extending from V3 to gp41 were exchanged are defective for growth in cultures of primary human macrophages. This defect appears to be macrophage-specific, since all recombinants replicate with comparable efficiency in peripheral blood lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00218-11-LMM

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and Chemical Studies on Retroviral DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Keith Peden	Expert	LMM, NIAID
	Klaus Strebel	Sr. Staff Fellow	LMM, NIAID
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SECTION

Biochemical Virology

INSTITUTE AND LOCATION

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PROJECT TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00300-12-LMM

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic aspects of viral oncogenesis in wild mouse species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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1.5	1	.5

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Various mouse chromosomal genes alter the susceptibility of mice to retrovirus-induced neoplastic disease. These genes include endogenous retroviral sequences as well as mouse cellular genes which facilitate or restrict virus replication. We have identified a novel common viral integration region associated with tumor induction by type-B leukemogenic retrovirus. This virus is related to mouse mammary tumor virus, but induces T-cell thymic lymphomas. A novel virus integration site was identified in 20% of the tumors screened, suggesting the presence of a functional gene involved in tumor induction. This gene was mapped to a site on the X chromosome not previously known to contain protooncogene sequences. Other experiments were designed to follow the genetic linkage of loci responsible for the clonal deletion of self-Mlsf reactive T cells. Results from these investigations indicated that at least three known MMTV proviruses, Mtv-8, -9, and -11 are responsible for this deletion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00301-12

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic mapping of mouse chromosomal genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of the effort to map and sequence the human genome, we have been involved in an ongoing effort to develop a multilocus genetic map of the mouse as the model mammalian system. Most of this work has been accomplished by the genetic analysis of the progeny of two genetic crosses, an interspecies and an intersubspecies backcross. DNAs from the progeny of these crosses have been typed for a variety of polymorphic reference loci to permit mapping of unknown markers to specific positions on the linkage map. These studies have resulted in the chromosomal mapping of a large number of genes including various genes expressed in nervous tissue, cyclin genes, heat shock proteins, transcriptional regulators, microtubule associated protein genes and homeobox genes. Several of these genes map at or near known mouse mutations and therefore are potential candidates for these mutations. Thus, one gene specifically expressed in brain was mapped near the coloboma neurological mutation and and shown to be deleted in mutant coloboma mice. Another gene encoding a major stuctural component of cartilage was mapped near the cmd (cartilage matrix deficiency) mutation, and cmd mice were shown to have a 7 b deletion of this gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AO 00304-12 LMM

PERIOD COVERED

October 1, 1992 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Retroviral Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Dr. K. Fujita	Visiting Associate	LMM, NIAID
	Dr. P. Dickie	Visiting Associate	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prior infection of cells with one retrovirus often renders them resistant to "super-infection" with another retrovirus. The resistance is thought to arise because viral envelope protein produced in the infected cell interacts with receptor, rendering it incapable of intereacting normally with incoming viruses. A similar mechanism may be responsible for certain forms of inherited resistance to retroviruses in animal systems in which the resistance is co-inherited with an expressed, "endogenous" retroviral envelope gene. One such endogenous retroviral envelope gene in mice genetically linked to a resistance trait (Fv-4) was cloned in LMM several years ago. To investigate the in vivo effects of this gene, we generated transgenic mice in which the Fv-4 envelope gene is driven by its associated flanking cellular promoter. Two transgenic strains were obtained. Southern blot analysis and genetic breeding experiments showed that each strain contains a transgene in a single (and distinct) location in the mouse genome. Northern blot analysis showed that the transgenes are expressed to high level in relevant lymphoid tissues. Susceptibility to retroviral infection In vivo was assayed by inoculating mice with Friend virus and scoring splenomegaly and spleen virus titer at various times after infection. Both transgenic mouse strains were markedly resistant to infection as assayed by XC test, although at high doses of inoculum some transgenic mice developed splenomegaly, indicating that the resistance was not absolute. This model of natural resistance to retroviruses could form the basis of "gene therapy" approaches to HIV. We are investigating the effects of placing other potential retroviral resistance genes, including the HIV envelope, downstream of the FV-4 promoter in tissue culture and transgenic systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00415 - 09

PERIOD COVERED

October 1, 1993 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M.A. Martin	Chief	LMM, NIAID
F. Maldarelli	Med.Staff Fellow	LMM, NIAID
L-J Chang	Visiting Associate	LMM, NIAID
R. Willey	Biologist	LMM, NIAID
P. Mounts	IPA	LMM, NIAID
R. Shibata	Visiting Fellow	LMM, NIAID

COOPERATING UNITS *(if any)*

John Brady	Microbiologist	LMV, DCE, NCI
Larry Arthur	Director, AVP	FCRDC, NCI

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The HIV-1 envelope glycoproteins, gp120 and gp41, mediate viral attachment and penetration into CD4+ cells; some isolates of HIV-1, however, are restricted in their ability to infect productively primary macrophages or continuous CD4+ cell lines. In order to study the biochemical basis for these differences, a variety of infectious molecular clones of HIV-1 containing defined mutations within the envelope glycoproteins were analyzed employing radiolabeled purified virions to evaluate envelope structure and function. The tendency of purified virions to release their gp120 envelope component either spontaneously or after interacting with a soluble form of CD4 increased in parallel with the ability of different HIV-1 variants to grow in CD4+ cell lines. In contrast, both spontaneous and soluble CD4-induced release of gp120 from virions of macrophage-tropic HIV-1 variants was substantially reduced. Work is currently in progress to determine the relationship between these altered physical and functional properties and the ability of HIV-1 to infect different cell types productively.

More than 90% of chimpanzees inoculated with HIV-1 have received derivatives of the HIV-1LAI/IIIB isolate. These inoculated chimps invariably become infected, produce antibodies to viral proteins, but fail to develop disease. In an attempt to develop a disease model of AIDS in HIV-1 infected chimpanzees, fresh HIV-1 isolates were screened from seropositive, symptomatic individuals for their ability to replicate in chimpanzee PBMCs. Of 23 samples freshly isolated from AIDS or ARC patients, 3 isolates (DH012, 20, and 29) could readily infect chimp PBMCs *in vitro*. DH012 and DH029 also readily infected human monocyte derived macrophages. DH012 replicated more rapidly and to higher titers in chimpanzee PBMCs compared to a "chimp-adapted" derivative of HIVIIIB. HIV-1 DH012 also induced marked CPE in both human and chimpanzee PBMCs and was able to infect PBMCs from 16 different animals including a sample from a chimpanzee that had been previously inoculated and continued to be persistently infected with HIV-1IIIB. The nucleotide sequence of the V3 envelope regions of the three "chimp-tropic" HIV-1 isolates were unremarkable. HIV-1DH012 (alone) and a mixture of the three chimp-tropic HIV-1 isolates will be used to infect chimpanzees.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00467-08

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Polypeptides Encoded by the HIV-1 Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S. Venkatesan Medical Officer LMM, NIAIDPI:

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sequence specific RNA binding is thematic of several regulatory mechanisms of HIV gene expression. HIV RNA capsidation and particle maturation are driven by similar biochemical events. Self-assembly of p55 Gag protein is sufficient to form the skeletal framework of the immature particle. Coexpression of the Gag-Pol fusion protein facilitated the maturation and processing as manifested by the condensation of the core. This morphologic change was accompanied by substantial Gag processing in the Gag-Pol particles as evidenced by the presence of abundant p24 (CA) protein and encapsidation of viral RNA. The cooperative interactions regulating capsidation and particle assembly are attractive for targeted drug development.

The mechanism of RNA encapsidation during retrovirus particle formation is still unclear. In particular, the nature and the identity of the RNA sequences and the gag subunits involved in HIV capsidation remain to be solved. We have now developed a simple in vivo model for HIV-1 RNA packaging. A subgenomic HIV RNA encompassing R, U5 and U3 elements surrounding the minimal packaging sequence was expressed from the T7 promoter using vaccinia T7 polymerase vector. Co-infection with GAG or GAG-POL recombinant vaccinia viruses lead to HIV particles that selectively packaged the mini HIV RNA. We have expressed cleavage defective mutants of GAG using the vaccinia vector. Mutations that eliminate the myristoylation or the nucleic acid binding CYS containig motif (NBCys) of the p9 subunit of GAG protein have been analyzed in the context of GAG-POL expression to examine whether they interfere with RNA packaging. This system is being used to define the molecular parameters of RNA capsidation. It is our eventual goal to develop molecular strategies to interfere with RNA packaging and particle maturation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00527-06

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Cloning and Characterization of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Theodore S. Theodore	Res. Microbiologist	LMM, NIAID
	George Englund	Biologist	LMM, NIAID
	Malcolm A. Martin	Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Heterogeneity among AIDS retroviral genomes is a distinct feature of lentiviruses. Because of this diversity, molecular clones provide the necessary molecular reagents for identifying and studying the functions of the viral genome. These clones can also be used to characterize viral determinants of cell tropism, cytopathicity, and virulence in cultured cells. Chimeric constructs between these molecular clones can be used to examine the function of individual genes as well as entry and replication. Our objective was to obtain information pertaining to the structure and diversity of HIV. Most HIV isolates cloned are T cell tropic. We have a complete molecular clone from a macrophagetropic viral isolate. Although T cells are the major target for HIV replication in peripheral blood, macrophages represent the predominant HIV -infected cell type in most tissues. Macrophages are probably the primary reservoir of HIV and sustain a persistent infection in individuals for many years. Our macrophagetropic clone (PAD-2), upon transfection into Hela cells followed by cocultivation on PBLs or macrophages, produced viral particles as measured by reverse transcriptase (RT) activity. This clone was unable to replicate in continuous T cell lines. Growth studies, as well as physical and biochemical analyses with this and other isolates, are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00528-06-LMM

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional Studies of HIV-1 Regulatory Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Venkatesan	Medical Officer	LMM, NIAID
Others:	H. S. Park	Visiting Fellow	LMM, NIAID
	Y. S. Nam	Visiting Fellow	LMM, NIAID
	S. Hiller	Visiting Fellow	LMM, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	4	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the life-cycle of HIV, the Rev protein regulates the temporal switch from the early regulatory to the late lytic phase. The Rev regulatory protein of HIV is a basic nuclear protein that concentrates in the nucleoli and activates the viral RNAs, by binding to a highly structured RRE (Rev Responsive Element) RNA . Our studies of Rev and RRE RNA were targeted to understand i) the minimum RRE sequence requirements for activation by Rev; ii) to explore the roles of RRE other than Rev binding in the activation process, iii) to analyze the various functional motifs of the Rev protein; and iv) to identify and characterize the function of the putative cellular factors that may bind RRE RNA, Rev, or both.

To examine the nature of the cooperative interactions between the RNA binding and protein oligomerization domains of Rev, we replaced the RNA binding motif of Rev with a poly-arginine tract both in the context of Rev and the Rev/MS-C fusion protein. Functionally defective of Rev/MS-C fusion protein mutants, that had lost the presumptive RRE RNA binding motif (residues 35-50), regained the activation potential for both RRE and MS2 RNA when 9 ARGs were inserted at the deletion. From these genetic studies, we propose that poly-arginine insertion near the N-terminus of Rev promotes nucleolar targeting in a context sensitive manner, and the Rev sequence immediately flanking the 9 arginines (residues 24-35, and residues 36-50) is required for RRE RNA binding.

We have cloned and characterized a HIV-1 Rev Responsive Element (RRE) RNA binding cellular factor (RBF) from a library of HeLa cDNA 1 coliphage recombinants. RBF bore significant sequence homology with many cellular and viral ds RNA binding proteins including the interferon inducible ds RNA activated protein kinase, PKR, and the vaccinia virus E3L protein. RBF was a competitive inhibitor of ds RNA activation of interferon induced PKR kinase *in vitro* and *in vivo*, and RBF expression in HeLa cells complemented the growth and protein synthesis defect of a vaccinia virus mutant deleted for the expression of the ds RNA binding protein, E3L.

PROJECT NUMBER

Z01 AI 00547-05 LMM

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanism(s) of Human Retrovirus trans-Regulatory Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	Kuan-Teh Jeang	Medical Officer, Research	LMM
Others:			
	A. Gatignol	Guest Researcher	LMM
	B. Dropulic	Visiting Associate	LMM
	O. J. Semmes, IV	Staff Fellow	LMM
	L. Huang	Visiting Associate	LMM
	K. Yim	Special Volunteer	LMM
	M. Martin	Chief	LMM

COOPERATING UNITS *(if any)*

Franca Majone, U. Padua, Italy

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the mechanisms by which HIV-1 Tat and HTLV-I Tax proteins activate transcription. Recent finding relevant to the HIV-1 system include the following: 1) We have obtained evidence that Tat functions as a transcriptional adaptor that indirectly activates the HIV-1 LTR only in the setting of upstream "enhancers." 2) We have implicated a novel non-transcriptional function in Tat that influences HIV-1 infectivity. 3) We have determined that differences in the basic RNA-binding domains of HIV-1 Tat and HIV-2 Tat confer type specific trans-activation of the respective LTRs. 4) We have defined the RNA-binding domain of a TAR-RNA-binding protein TRBP, which categorizes this protein into the family of RNA-binding proteins that includes human dsI kinase and Drosophila Staufen.

For the HTLV-I system, recent accomplishments include: 1) the demonstration that HTLV-I Tax protein induces the formation of micro-nuclei in mammalian cells. 2) The characterization of an activation domain and a targetting domain in Tax.

A third area of interest for the laboratory is the study of ribozymes. We have constructed five different ribozymes each targetted to an independent locus in the HIV-1 genome. We have also made transgenic mice that contain ribozyme-genes which direct synthesis of ribozymes capable of cleaving HIV-1 RNAs. Ongoing studies in the laboratory have also delineated that ribozymes can be used as thermal stable enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00669-01

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Retroviral Genes and their Products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Klaus Strebel	Senior Staff Fellow	LMM, NIAID
	Frank Maldarelli	Research Associate	LMM, NIAID
	Mao-Yuan Chen	Guest Researcher	LMM, NIAID
	Ulrich Schubert	Guest Researcher	LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

HIV is a complex retrovirus containing a number of genes not commonly found in other retroviruses. One of these genes, vpu, which is encoded only by HIV-1 and does not have any known homologues in HIV-2 or SIV, encodes for a small integral membrane protein. We have in the past identified two biological activities for Vpu: (i) enhancement of particle release; (ii) degradation of CD4. To study the effect of Vpu on particle release, we introduced a series of mutations into the infectious molecular clone, pNL43, eliminating the Vpu gene and/or the Env gene. Mutant constructs were analyzed in transient expression assays in HeLa cells by pulse/chase analyses for their ability to produce and secrete virus particles. We found that the presence of Env is not required for HIV-1 particles to be secreted into the culture medium. In fact, particle release was similarly Vpu dependent in the presence or absence of Env. This indicates that Vpu enhances particle release through a mechanism that does not involve the Env product.

To study the mechanism of Vpu-induced CD4 degradation, we developed an in vitro assay which involves the expression of Vpu and CD4 in rabbit reticulocyte lysates in the presence of canine microsomal membranes. The advantage of this in vitro system over a whole cell system is its simplicity and accessability for experimental variations. We constructed a series of in vitro expression plasmids that allowed us to synthesize wild-type CD4 or Vpu or specific mutants of both proteins in vitro. We found that Vpu can indeed induce degradation of CD4 in vitro. Successful CD4 decay required the presence of Vpu and CD4 in the same membrane compartment. Co-expression of CD4 and Vpu in rabbit reticulocyte lysate in the absence of microsomal membranes did not result in any appreciable destabilization of CD4. Analyses involving specific mutants of CD4 and Vpu indicate that (i) glycosylation of CD4 is not a prerequisite for degradation; (ii) CD4 decay occurs post-translational; (iii) Vpu targets a short sequence in the cytoplasmic tail of CD4; (iv) degradation in vitro is significantly slower than in vivo, yet there are no degradation intermediates detectable; therefore, (v) CD4 decay is likely to be a multi-step process; (vi) short deletions from the C-terminus of Vpu or an internal highly conserved domain result in a loss of biological activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00689-01

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A genetic and biological analysis of HIV-1 and HIV-2

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. Peden	Visiting Scientist	LMM, NIAID
Others:	M. Ryan	Guest Researcher	LMM, NIAID
	R. Willey	Biologist	LMM, NIAID
	M. Martin	Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

Orit Nahor Hadassah Medical School, Jerusalem, Israel

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Virus derived from an infectious molecular clone of ELI was found to be infectious in PBMC, was found to exhibit a delayed time course of infection on CEM and H9 cells, but was unable to infect U937 cells. The virus that arose from the H9 cells was able to infect CEM and H9 cells without any delay in replication kinetics. In addition, this passaged virus was able to infect U937 cells. Two regions in the envelope gene were found to have mutations, and these changes were shown to confer the enhanced replicative capacity and expanded host range characteristics of the passaged virus. A Val-7 substitution in gp41 conferred the ability to replicate in T cell lines, while the additional Arg-427 change in gp120 was required before the virus could replicate in the promonocytic U937 cell line. The effect of these mutations on several parameters of envelope structure has been investigated. We have determined that the Met-7 to Val substitution in gp41 does not have a significant effect on spontaneous gp120 shedding although it increases the affinity of virions for CD4-IgG and amount of the CD4-induced dissociation of gp120 from virions. With both the gp41 Val-7 and the gp120 Arg-427 substitutions, the affinity of the virions for CD4-IgG, the spontaneous gp120 release, and the CD4-induced gp120 shedding are increased further. Thus, there is a correlation with the ability of a virus to replicate in cell lines and the tendency of its envelope to release gp120 after association with CD4.

Mutations were constructed in the *nef* genes of several strains of HIV-1 and HIV-2 in order to ascertain the effect of Nef on virus replication *in vitro*. The effect of Nef on virus replication was more variable when tested on various CD4-positive cell lines. In some cases, there was no detectable difference in the replication kinetics between the Nef mutant and wild type viruses. These results demonstrate that the effects of Nef are variable and depend on the particular host-virus system, and that Nef is usually a positive factor for the replication of HIV *in vitro*.

LABORATORY OF MALARIA RESEARCH
1993 Annual Report
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Laboratory of Malaria Research
National Institute of Allergy and Infectious Diseases
Summary - October 1, 1992 - September 30, 1993

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

The Laboratory of Malaria Research has completed its first full year as an independent unit. However, we still interact closely with the Laboratory of Parasitic Diseases, sharing a common equipment room, a joint weekly seminar series and several joint research efforts. Dr. D. Sacks of the Laboratory of Parasitic Diseases works on *Leishmania* in sandflies in the LMR insectary. Dr. T. Wellems continues to serve as coordinator of LMR clinical programs and liaison with Dr. Neva and the LPD clinical service.

The LMR involvement with the Malaria Research and Training Center of the National School of Medicine and Pharmacy in Mali continues as our primary international activity. Support for this program comes from the U.S. Agency for International Development, the WHO and the Rockefeller and MacArthur Foundations. Dr. R. Sakai is the NIH Resident Scientist in Bamako, and is responsible for coordinating activities between Mali and U.S. collaborators. Dr. S. Dolan departed the Genetics and Pharmacology Section of the LMR in May after six years at the NIH, has taken an appointment in the Department of Tropical Medicine at Tulane, and has been posted full time at the Malaria Center in Mali. Dr. C. Plowe was able to evaluate the PCR-based test for the cycloquanil resistant Thr - 108 mutation in *Plasmodium falciparum* in Mali where proquanil is being used. In the future, the Malaria Center will serve as the focus for field studies in collaboration with Malian scientists on the population biology of *Anopheles gambiae*, the search for mechanisms of refractoriness to malaria in vector populations, and the development of microbial insecticides for vector control.

This has been an exciting period for research on malaria ligands and receptors for invasion of erythrocytes. Drs. C. Chitnis and L. Miller working with Dr. Horuk of Genentech have shown that the Duffy blood group determinant, the receptor for *P. vivax*, is the erythrocyte chemokine receptor. The chemokines, IL8 and MGSA, completely block invasion, suggesting the possibility of receptor blocking therapy for malaria. Drs. C. Chitnis, K. Sim and L. Miller have identified the domain on the Duffy binding ligand of *P. vivax* and *P. knowlesi* and on the sialic acid binding ligand of *P. falciparum* for binding erythrocytes. This work has identified an important parasite domain that has been shown to be present in repeated copies in the malaria genome by the Section headed by Dr. T. Wellems in our laboratory. This domain well serve as a new focus for vaccine research designed to block important steps in the host-parasite interaction and aid in receptor blocking therapies.

Drs. G. Ward, J. Dvorak and L. Miller have shown that the parasitophorous vacuole derived from the host cell membrane, not the parasite. This indicates that only the parasite induces the formation of the vacuolar membrane.

The Molecular Biology Section under Dr. T. McCutchan has produced attenuated lines of human and rodent malarias and studied their developmental characteristics. Drs. G. McConkey and T. McCutchan have defined auxotrophic mutants that are characterized by their dependency on either exogenous pyrimidines or para-aminobenzoic acid (PABA). With Drs. S. Meshnick at Michigan, G. McConkey and T. McCutchan have shown that the PABA-dependent mutants do not synthesize PABA as does their wild type counterpart. In the presence of PABA, both mutant and wild type parasites have equivalent growth curves, produce gameteocypes and can be passaged through mosquitoes. Drs. D. Kaslow, G. McConkey, T. McCutchan and Mr. D. Keister are also studying the complementation of yeast mutants with genes derived from malaria parasites in an effort designed to characterize genes like PABA synthetase but also with an eye to other genes like those encoding proteins involved in drug resistance, development and sexual differentiation.

Drs. X. Su, V. Heatwole and T. Wellems have shown that a 200 kb segment of chromosome 7 of *P. falciparum* is linked to chloroquine response. About 30 kb of sequence have been obtained from this segment and we anticipate 50-100 kb by the end of 1993. Mutations and gene rearrangements are being tested for linkage to chloroquine response among geographically distant parasites.

Sequencing studies by Drs. D. Peterson, X. Su and T. Wellems of the chloroquine resistance (CQR) locus have identified numerous *P. falciparum* open reading frames (ORFs) with homology to proteins involved in red cell invasion. Alignments are strongest to conserved cysteine-rich domains in the *P. falciparum* sialic acid binding protein (SABP), EBA-175, and the *P. knowlesi*/*P. vivax* Duffy receptor family. We have used conserved sequences from these new *P. falciparum* ORF's to isolate additional genes that may be involved in red cell invasion.

The dihydrofolate reductase (DHFR) Asn-108 mutation has been linked to pyrimethamine resistance in Kenya, and recent date indicate that the cycloguanil resistance Thr-108 mutation is present in Mali where proguanil is being used. Data from surveys in these countries may provide a basis for regional adjustment of therapeutic strategies in health programs (Drs. W. Watkins, C. Plowe, and T. Wellems).

Drs. K. Creedon working with Dr. T. Wellems was recently awarded the Ph.D. from the Catholic University of America for showing that *P. falciparum* S-adenosylhomocysteine hydrolase can be expressed in *E. coli*. Kinetic and functional comparisons with mammalian AHCY will now be possible.

Two cytoplasmic genomes (6 kb mitochondrial-like; 35 kb chloroplast-like) were shown maternally inherited in *P. falciparum* by Drs. A. Vaidya and T. Wellems. In 16 independent recombinants of the HB3xDd2 cross, the cytoplasmic elements were inherited from Dd2 parent only. Defective exflagellation of Dd2 male gametes has been demonstrated. The genetic locus carrying this exflagellation defect is being mapped by linkage analysis.

Efforts are being directed to the transient transfection of *P. falciparum* erythrocytic stage parasites by Mr. C. Sifri, a Howard Hughes Fellow, and Dr. T. Wellems. Luciferase and CAT reporters have been incorporated into plasmid constructs with control regions of the PfHRP-II genes. Ballistic methods, lipofection and electroporation are being evaluated for DNA delivery.

Experiments by Drs. E. Mialhe and L. Miller using a modification of the biolistic system have produced high levels of transient expression of markers in mosquito embryos. Various constructs are now being evaluated for their abilities to facilitate stable integration.

Using *in vitro* cultures of *P. gallinaceum* ookinetes and oocysts, Dr. A. Warburg is now pursuing studies to identify the basement membrane component(s) to which ookinetes adhere. Preliminary results indicate that ookinetes adhere very avidly to mouse laminin and collagen I.V. Furthermore, oocysts also adhere to Pronectin-F, a synthetic protein polymer containing multiple copies of the universal integrin recognition sequences R-G-D. Dr. A. Warburg plans to use available peptides derived from laminin and collagen IV to idenify a sequence with higher affinity. Once defined, such a peptide may be used to pull out and characterize a receptor of ookinetes and oocysts. Such receptors may also be present on sporozoites that need to attach to and traverse the basement membrane of the salivary glands.

Mr. D. Keister and Dr. D. Kaslow reported on a technique for cryopreservation of *P. falciparum* gametocytes permitting the long term storage of quanitites of material infectious to mosquitoes when required.

Drs. K. Williamson and D. Kaslow cloned and sequenced the complete 9.4 kb open reading frame gene that encodes the 360/310-kDa transmission-blocking target antigen, Pfs230. The repeat region present at the amino terminal end of the 360-kDa form of Pfs230 appears to be cleaved off at the time of emergence and exflagellation to give rise to the 310-kDa surface form of Pfs230. In an attempt to recreate the native structure of Pfs230, recombinant protein produced in eukaryotic systems are now being studied as potential vaccines.

Drs. M. Shahabuddin and D. Kaslow have shown that mosquito produced protease(s) activates parasite produced chitinase which is involved in penetration of the pentrophic membrane. Addition of exogenous chitinase or polyoxin D (an inhibitor of chitin synthase), two compounds that disrupt the peritrophic membrane, reversed the transmission-blocking effects of allosamidin and leupeptin. The parasite chitinase is now being cloned as a potential target of transmission-blocking immunity.

Drs. P. Duffy and D. Kaslow cloned *P. falciparum* Pfs28, the analogous gene to that encoding Pgs28 (a 28-kDa surface protein of *P. gallinaceum* ookinetes that is a target of transmission-blocking antibodies). The deduced amino acid sequence of Pfs28 was found to be similar to that of other transmission-blocking target antigens that consist predominantly of four cysteine-rich (EGF-like) domains. However, comparison of these and other proteins now clearly suggest that there are at least two subfamilies of EGF-like ookinete surface proteins. Expression of Pfs28 in yeast is in progress.

Drs. M. Fried and D. Kaslow have isolated gametes and ookinetes of *P. knowlesi*, a primate malaria parasite closely related to *P. vivax*. Using a panel of monoclonal antibodies developed against surface proteins of *P. knowlesi*, two cysteine-rich, lipophilic surface proteins of 20-and 24-kDa have been identified and purified by two-dimensional gel electrophoresis in amounts sufficient to attempt protein microsequencing. This will identify the sequences for cloning these genes.

Dr. D. Kaslow has been developing Pfs 25 for human phase 1 trials. Both alum-adjuvanted and microencapsulated, yeast-produced Pfs25 have been shown to induce complete transmission-blocking activity in laboratory animals. Due to a lack of private industrial support, a yeast expression system has been established in the laboratory. This system has been used to express a number of constructs designed to secrete histidine-tagged recombinant Pfs25. Fermentation conditions and purification processes have been determined that allow for production of sufficient quantities of highly purified recombinant Pfs25 for clinical trials.

Dr. M. Touray has shown salivary gland sporozoites are more infectious to the vertebrate host than oocyst sporozoites. To determine the physiologic basis of these developmentally regulated sporozoite infectivities, he analyzed their relative susceptibilities to lysis by vertebrate host serum. Dr. Touray observed that the noninfectious oocyst sporozoites are 4 times more susceptable to lysis by serum than the infective salivary gland sporozoites, indicating that one factor in the development of infectivity to the vertebrate host is resistance to lysis by vertebrate host serum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00108-22 LMR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Studies on the Immunobiology of Malaria

PRINCIPAL INVESTIGATOR	L. H. Miller	Head, Malaria Sec.	LMR, NIAID
	S. Kumar	Visiting Associate	LMR, NIAID
Others:	T. McCutchan	Senior Scientist	LMR, NIAID
	T. Jing-Hui	Visiting Fellow	LMR, NIAID
	J. Gorden	Biologist	LMR, NIAID
	D. Kaslow	Senior Staff Fellow	LMR, NIAID

COOPERATING UNITS *(If any)*

J.A. Berzofsky, Sr. Scientist, Metabolism Branch, NCI; R. Houghten, M. So, and F. Heffron, Univ. of Oregon, Portland; M.F. Good, QIMR, Brisbane, Australia; A. Agar, U. of Miami; W.E. Collins, CDC, Atlanta, GA

LAB/BRANCH

Laboratory of Malaria Research

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Immunity to asexual erythrocytic parasites in certain rodent malarias is dependent on cell mediated mechanisms that are dependent on CD4$^+$ T cells and the spleen and are independent of antibody. Such immunity can be induced by Salmonella and malarial antigens. The present studies are designed to identify those proteins and T cell epitopes that will lead to protection. In addition, we are developing studies to test constructs in Salmonella and BCG that will lead to protection against *Plasmodium falciparum* in *Aotus* monkeys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00208-13 LMR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR	Thomas F. McCutchan	Microbiologist
Others	G. McConkey	Senior Staff Fellow
	Y. Lee-Wickner	Technician
	Jun Li	Guest Worker

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Malaria Research

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The Molecular Biology Section has produced attenuated lines of human and rodent malarias and studied their developmental characteristics. We have defined auxotrophic mutants of both species of parasite as characterized by their dependency for growth upon either exogenously supplied pyrimidines or para-aminobenzoic acid. These should prove useful in the study of both the biochemistry of parasites and the development of immunity to parasites. We are also studying the complementation of yeast mutants with genes derived from malaria parasites in an effort designed to characterize genes involved in drug resistance, development, and sexual differentiation.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00240-12 LMR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D.C. Kaslow, Senior Staff Fellow, LMR, NIAID

Others: K. Williamson, Guest Researcher, LMR; M. Shahabuddin, Visiting Fellow, LMR; M. Fried, Visiting Fellow, LMR; P. Duffy, Guest Researcher, LMR; D. Keister, Biologist, LMR; O. Muratova, Special Volunteer, LMR; R. Hearn, Entomologist, LMR; M. Criscio, Student Trainee Biol., LMR

COOPERATING UNITS *(if any)*

See attached

LAB/BRANCH

Laboratory of Malaria Research

SECTION

Molecular Vaccine Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL.	OTHER
8.3	5.0	3.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Four potential target antigens (Pfs25/Pgs25, Pfs28/Pgs28, Pfs40, and Pfs230) of transmission-blocking antibodies have now been cloned in our laboratory, and a fifth (a parasite-produced chitinase) and a sixth (a mosquito-produced protease) have been identified. Of the four target antigens that have been cloned, all have been expressed in one or more recombinant expression systems, but only recombinant Pfs25 (rPfs25) has induced transmission-blocking antibodies in laboratory animals. Our immediate goals are to 1) test in humans the safety, immunogenicity, and efficacy of a Pfs25 subunit vaccine and design a means of testing the efficacy of such a transmission-blocking vaccine in the field, 2) express Pfs28 as a recombinant protein in a form that elicits transmission-blocking antibodies and test various combinations of rPfs25 and rPfs28 in a cocktail vaccine to determine if the combination elicits longer lasting or higher titer transmission-blocking antibodies, 3) determine the role, if any, that the calcium-binding Pfs40 plays in sexual development and ascertain if Pfs40 is a target of transmission-blocking antibodies, 4) identify and express as recombinant protein the B-cell epitopes in Pfs230 responsible for the transmission-blocking activity of our monoclonal antibodies to Pfs230, 5) determine if antibodies to parasite-produced chitinase or mosquito-produced protease completely block transmission, and if so, isolate and express the genes encoding these enzyme, and 6) isolate analogous genes to these five parasite proteins, if they exist, from *P. vivax*. Our more long term goals include identifying new target antigens on sexual stage parasites, and defining the molecular mechanisms involved in fertilization of malarial parasites.

PHS 6040 (Rev. 5/92)

Cooperating Units:

J. Coligan, Branch Chief, BRB, NIAID; B. Moss, Lab Chief, LVD, NIAID; J. Shiloach, Unit Chief, LCBD, NIDDK; Chiron Corporation, Emeryville, CA (Jim Merryweather) Wyeth Laboratories, Radnor, PA (P. Hung); Catholic University, Nijmegen, The Netherlands (J. Schoenmakers and R. Sauerwein); Pasteur Institut, Paris, France (G. Langsley); Scripps Clin. Res. Fnd., La Jolla, CA (A. Satterwaith), E. Stura, A. Kang); CDC, Atlanta, GA (B. Collins); US Army (Walter Reed Army Institute of Research, Washington, D.C. -R. Ballou and J. Sadoff); Case Western Reserve School of Medicine, Cleveland, OH (M. Aikawa), Virogenetics Corp., Troy, NY (E. Paoletti), SmithKline and Beecham, King of Prussia, PA (M. Gross), Immunex Corp., Seattle, WA (G. Price).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00241-12 LMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR	L. H. Miller	Laboratory	LMR, NIAID
Others:	C. Plowe	Staff Fellow	LMR, NIAID
	D. Hudson	Microbiologist	LMR, NIAID
	C. Chitnis	Visiting Fellow	LMR, NIAID
	G. Ward	Visiting Associate	LMR, NIAID
	S. Dolan	Medical Staff Fellow	LMR, NIAID
	K. L. Sim	Expert	LMR, NIAID

COOPERATING UNITS *(if any)*
F. Klotz and J.D. Haynes, WRAIR; M. Aikawa, Case Western Reserve University; T. Hadley, University of Louisville; R. Horuk, Genentech.

LAB/BRANCH
Laboratory of Malaria Research

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The merozoite interacts in a receptor-specific manner with the erythrocyte surface and is the stage upon which immunity may work to block invasion. Thus, merozoite surface components are of interest because of their role in erythrocyte recognition and as antigens for induction of protective immunity. We are identifying *P. knowlesi, P. vivax* and *P. falciparum* receptors for attachment to monkey and human erythrocytes. The components in the junction and the signaling after merozoites make contact with erythrocytes is also under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00248-12 LMR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes

PRINCIPAL INVESTIGATOR	L. H. Miller	Laboratory Chief	LMR, NIAID
	R. W. Gwadz	Section Head	LMR, NIAID
	K. D. Vernick	Senior Staff Fellow	LMR, NIAID
Others:	D. C. Kaslow	Medical Staff Fellow	LMR, NIAID
	M. Shahabuddin	Visiting Fellow	LMR, NIAID
	A. Warburg	Visiting Associate	LMR, NIAID
(See below)	M. G. Touray	Visiting Fellow	LMR, NIAID

COOPERATING UNITS *(if any)*

National School of Medicine, Bamako, Mali (Dr. Y. Toure); Oak Ridge National Laboratory (Dr. P. Mazur); Case Western Reserve University (Dr. M. Aikawa)

LAB/BRANCH

Laboratory of Malaria Research

SECTION

Molecular Entomology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
4.9	3.2	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Others (Cont.): E. Miahle, Guest Researcher, LMR, NIAID; M. Valencia, Special Volunteer, LMR, NIAID; D. Seeley, Supervisory Entomologist, LMR, NIAID; P. Pimenta, Guest Researcher, LPD, NIAID

Other Cooperating Units: Biological Laboratories, Harvard Univ. (Drs. F. Kafatos and B. Zheng)

The biology of *anopheline* mosquitoes is being studied in relation to the capacity of these vectors to transmit malaria. Genetic, molecular, biochemical and immunological studies are seeking to describe mechanisms in the mosquito which enhance or retard development of the malaria parasite with emphasis on the behavior of ookinetes, oocysts and sporozoites.

To facilitate these studies 1) techniques have been developed to cultivate the sporogonic (mosquito) stages of the malaria parasite *in vitro*; 2) methods are being improved for cloning and transposing genes into mosquito germ lines; and 3) systems are being evaluated for the cryopreservation of mosquito embryos to increase our capacity to store genetic material.

The ability to identify, clone, and transpose genes which regulate mechanisms which render mosquitoes refractory to parasite development should facilitate development of control strategies based on the introduction into the field of mosquito populations incapable of transmitting malaria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00483-08 LMR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Molecular Genetics of Drug Resistance and Red Blood Cell Invasion in Malaria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas E. Wellems	Section Head	LMR, NIAID
Others:	David Peterson	Senior Staff Fellow	LMR, NIAID
	Stephen Dolan	Medical Staff Fellow	LMR, NIAID
	Xin-zhuan Su	Senior Staff Fellow	LMR, NIAID
	Kathleen Creedon	Guest Researcher	FDA
(see attached)	Virginia Heatwole	Research Fellow	LMR, NIAID

COOPERATING UNITS *(if any)*

Sloan Kettering Institute, New York (Dr. J. Ravetch); Catholic University, Washington, D.C. (P. Rathod)

LAB/BRANCH

Laboratory of Malaria Research

SECTION

Genetics and Pharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL	OTHER:
7	4.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Molecular basis of chloroquine resistance. Genetic analysis has indicated that chloroquine resistance in *P. falciparum* is controlled by a single gene or closely linked group of genes within a 200 kb segment of the parasite's seventh chromosome. We are isolating transcribed sequences from this segment and evaluating them as candidate chloroquine resistance genes.

Other drug development targets. Studies have progressed on two *Plasmodium* enzymes: S-adenosylhomocysteine hydrolase (AHCY) and dihydrofolate reductase-thymidylate synthase (DHFR-TS). The *P. falciparum* AHCY has been expressed in *E. coli* for kinetic and functional comparisons with mammalian AHCY. Knowledge of point DHFR-TS mutations that produce resistance to pyrimethamine and cycloguanil is being used in PCR assays for epidemiological studies in Kenya and Mali.

Transfection of *Plasmodium* erythrocytic stages. Efforts have focused transient expression experiments using luciferase and chloramphenicol-acetyl transferase (CAT) reporters flanked by control regions of the *P. falciparum* HRP-2 AND HRP-3 genes. Ballistic methods, lipofection and electroporation are being evaluated for DNA delivery.

Genetic analysis of red cell invasion. A family of *P. falciparum* genes has been identified that encodes conserved domains known to be involved in red cell invasion. One of these genes (*ebl*-1) maps to a subtelomeric region of chromosome 13 that has been implicated in the efficiency of invasion. Preliminary evidence suggests that changes in expression of certain *ebl* genes may be associated with a switch between sialic acid-dependent and sialic acid-independent invasion pathways.

Cytoplasmic inheritance in P. falciparum Unidirectional dominance in the inheritance mitochondrial-like and plastid-like genomes has been found in two genetic cross. Defective differentiation of male gametes occurred in a parent of one cross and is being mapped by linkage analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00629-02 LMR

PERIOD COVERED
October 1, 1993 to September 30, 1993

TITLE OF PROJECT
Population Biology of *Anopheles gambiae* in Africa

PRINCIPAL INVESTIGATOR	R. W. Gwadz	Section Head	LMR, NIAID
	R. K. Sakai	Senior Scientist	LMR, NIAID
Others:	K. D. Vernick	Senior Staff Fellow	LMR, NIAID
	T. McCutchan	Section Head	LMR, NIAID
	K. Mathiopoulos	Visiting Fellow	LMR, NIAID

COOPERATING UNITS *(if any)*
National School of Medicine, Bamako, Mali (Dr. Y. Toure); Inst. of Parasitology, Univ. of Rome (Dr. Mario Coluzzi); Univ. of Maryland Biotechnology Inst. (Dr. R. Colwell)

LAB/BRANCH
Laboratory of Malaria Research

SECTION
International Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
2.6	2.3	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Others (Cont.): G.C. Lanzaro, Guest Researcher, LMR, NIAID; D. Bryant, Guest Researcher, LMR, NIAID; J. Li, Guest Researcher, LMR, NIAID: J.G. Estrada, Guest Researcher, LMR, NIAID

Other Cooperating Units: Biological Laboratories, Harvard University (Dr. F. Kafatos and B. Zheng)

Anopheles gambiae s.s. is the primary mosquito vector of malaria in Africa. As a prelude to the evaluation of strategies for malaria control based on the concept of replacement of vector populations with mosquitoes unable to transmit the malaria parasite, we are examining, in depth, the genetic and biologic structure of vector populations in a series of distinct ecological zones in Mali (West Africa). To facilitate these studies, we are using the tools of molecular genetics directed at microsatellite polymorphisms, restriction length polymorphisms (RFLPs), and ribosomal and mitochondrial DNA. Field studies of vector populations using these techniques will be conducted at the Malaria Research and Training Center recently established in Bamako, Mali, in collaboration with staff of the National School of Medicine and Pharmacy of Mali. In addition, studies on larval ecology and nutrition of *An. gambiae* in Mali seek to identify microorganisms in the diet which might serve as carriers of genes coding for the production of biologically active insecticides for use in larval control programs.

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
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Annual Report
LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1992, to September 30, 1993

RESEARCH HIGHLIGHTS

LMSF scientists study disease-causing microbes and their interactions with eukaryotic hosts. General goals are to define the infectious strategies and mechanisms employed by the pathogens and the host responses that might be exploited to prevent or treat infections. Special attention is directed toward genetic events responsible for microbial variations, especially as they relate to avoidance/evasion of host defenses.

During FY 1993 LMSF scientists focused their research efforts on *Neisseria gonorrhoeae* (Gc), *Borrelia burgdorferi* (Bb), group B streptococci (GBS), *Pseudomonas aeruginosa*, and human immunodeficiency virus (HIV). Other studies in LMSF employ better-defined systems to explore basic aspects of parasitism (including conjugal genetic transfers), microbial mutation in response to their environment, and molecular aspects of host defense. Antibodies directed against surface-exposed viral or bacterial antigens and conjugated to ricin, streptonigrin, or other eukaryotic cell toxins are being constructed and evaluated for possible utility in treating infections.

Neisseria gonorrhoeae: Previous studies by LMSF scientists established the importance of pili and outer membrane Opa proteins in genesis of acute gonorrheal infection of males. Both these Gc surface components exhibit striking variability *in vitro* and *in vivo* following insertion of "new" nucleotide stretches into the *pil*E gene that encodes the pilin protein subunit. Stuart Hill explored contributions of DNA-transformation to pilus variation; he found that transformation with DNA containing a novel *pil*E efficiently alters recipient *pil*E sequence if donor molecules contain both 5' and 3' or just 5' flanking homologies. Such transfer probably mediates spread of a particular *pil*E sequence through populations of piliated Gc, but the results argue that such a mechanism does not account for the kinds of variation typical for *pil*E of Gc *in vitro* and *in vivo*. This contention is supported by finding that transformation-deficient Gc exhibit *pil*E variation similar to that of wild-type, transformable Gc. Bob Belland is defining the structural features of *opa* genes and the "illegitimate" recombinational events that account for their "phase variation" (on/off switching) and subsequent changes in expression of the outer membrane Opa proteins they encode. His results show that *opa* structural features (presence/absence of -35 control regions, number of pentameric -CTTCT- repeats in triplex-forming, leader-encoding regions), recombination and DNA repair enzymes (*mut*S, *mut*L), *opa* transcription levels, and DNA supercoiling all influence the frequencies at which individual *opa* genes undergo switching. Belland has constructed recombinant *opa* genes, some being mosaics with elements from more than one opa and other having site-specific mutations, which are expressed in *E. coli* to define which portions of Opa proteins mediate the biologic activities typical of Opa$^+$ Gc. These *opa* genes have been introduced into *E. coli* expressing different lipopolysaccharide (LPS) moieties (in collaboration with John Klena) to evaluate their biological affects in different LPS contexts. Klena has begun an genetic analysis of the biosynthetic components involved in synthesis of ligooligosaccharide (LOS) by Gc. In collaboration with Tie Chen, Belland has examined the behavior of Gc and recombinant *E. coli* expressing different Opa proteins as per their adherence to and internalization by tissue culture cells. Promoted adherence of Opa$^+$ bacteria holds for tissue culture cells of both human and non-human origins, in contrast to the human cell-specific adherence of Gc that is attributable to their possessing pili. Other studies of Chen's involve killing of Gc by sera from normal humans. He and Steve Fischer find that LOS phenotype is a dominant determiner of serum-resistance for these organisms, but sialylation of LOS, Opa phenotype, and piliation status also exert affects. Chen found that preincubation of Gc in urine from normal male volunteers renders Gc of certain phenotype combinations totally resistant to killing by serum. Similar affects are found for Gc preincubated with heparin or DNA (to a lesser extent); the mechanism is currently being elucidated. Fischer has preliminary findings that a surface-exposed region common to all Opa proteins mediates interactions between Gc and human neutrophils, *in vitro*. John Swanson has found that pilus$^+$ Gc exhibit less negative (i.e. more positive) surface charge than pilus$^-$ variants, as deduced by the electrophoretic mobilities of whole, intact cells. The extent to which surface charge is altered by pili correlates with which pilin subunits are expressed. The charge of pilus$^+$ Gc closely resembles that of isolated pili and is not a consequence of "viscous drag" as proposed by others. The influence of pili on surface charge largely overrides charge attributable to LOS, Opa expression, or accretion of polyanions by certain Opa$^+$ organisms.

Immunoglobulin biology: Seth Pincus formulated and constructed a variety of immunotoxins based on conjugating ricin A chain to either polyclonal or monoclonal antibodies that are effective against HIV infection *in vitro*. He found that addition of soluble CD4 greatly accentuated the killing of HIV-infected tissue culture cells by immunotoxins directed against gp41. Variants of HIV and of the host cells that resisted immunotoxin action are being analyzed by Pincus along with Tom Duensing and Hua Fang to localize the relevant epitopes or processes that allow their "escape" from killing by the immunotoxins. In addition, Pincus (in collaboration with L. Evans, LPVD), has constructed immunotoxins based on antibodies directed against MuLV, has demonstrated their effectiveness *in vitro*, and is assessing their efficacy *in vivo* against MuLV infection of mice and their associated neurodegeneration and neoplasia. Initial results indicate that virus infected cells are killed *in vivo*. Pincus and Vladimir Tolstikov are designing and constructing other immunotoxins that conjugate other toxic moieties (such as streptonigrin) through HIV protease-cleavable linkers to immunoglobulin molecules. Pincus and Carol Horgan used recombinant techniques to generate immunoglobulin molecules with deleted or modified "hinge" regions to examine the affects of hinge changes on antigen binding and complement activation. Pincus has also discovered colony variants (opaque/transparent) of group B streptococci that have differing virulence properties in mice and occur in organisms freshly isolated from human infections.

***Borrelia burgdorferi*:** Studies continued in LMSF on variation in outer surface proteins and on adaptive responses of the spirochetes causative of Lyme disease. Patricia Rosa has defined a number of structural/antigenic variants of the OspA and OspB surface proteins that result from point mutations or recombinational deletions in their highly homologous, tandemly-arrayed genes. Rosa and Neil Margolis found another variant phenotype in which OspA and OspB are not expressed though their respective genes are intact and functional in *E. coli*. This has led to their study of transcriptional regulation of ospA/ospB and the search for relevant DNA-binding proteins. Kit Tilly identified, cloned, and sequenced *B. burgdorferi* genes homologous to *dna*K, *dna*J, and *grp*E "heat-shock" elements of *E. coli*; she also identified *ihf* and *rho* gene homologues in the spirochete. Tilly has compelling evidence for linear plasmids replication intermediates with fused ends, adding new information on the possible modes of replication for these interesting structures. She also continued her quest to devise a method for introducing DNA into *B. burgdorferi*. Visiting workers Sherwood Casjens and Wai Mun Huang (University of Utah) completed a preliminary map chromosome that was largely done during their FY'92 summer stint in LMSF.

Microbial sex: Jack Heinemann continued studying several aspects of genetic exchange between microorganisms. First, he found that DNA transfer is not inhibited by gyrase inhibitors, so bacteria killed by such antibiotics retain their potential to be donors of DNA to new bacterial hosts. Second, he demonstrated that conjugation was unidirectional, and any reverse flow of genetic information required conversion of donors into recipients. Third, Heinemann showed that studies on adaptive mutagenesis (e.g. from leucine auxotrophy) can be complicated by occurrence of mutants that gain an ability to concentrate extracellular nutrient (e.g. leucine).

***Pseudomonas* iron acquisition:** Bob Ankenbauer isolated and sequenced a gene that encodes a iron-siderophore binding outer membrane protein of *P. aeruginosa*. This pyochelin receptor has little structural homology to other moieties that bind similar siderophores in different bacteria. The *fpt*A gene's control region resembles analogous genes in *E. coli* in containing a Fur-binding site, but the Fpt protein has no TonB box which is a conserved element of other iron-siderophore receptors. Ankenbauer and Cheryl Dooley have identified cosmid clones containing pseudomonas DNA that complement cells defective in siderophore biosynthesis. These will be used to define more precisely the actual components whose absences produce those biosynthetic defects.

Antimicrobial genes of macrophages: Kolari Bhat has constructed cDNA libraries from human macrophage cell line so as to identify genes whose expression is specific to macrophages and whose products have antimicrobial activities. Bhat has obtained partial or complete sequence on a large number of genes that likely have "housekeeping" functions in the macrophages. In addition, he has discovered previously undescribed genes that include: a superoxide dismutase gene; a gene with considerable homology to nematode chitinases; a proteinase inhibitor; and a tyrosine kinase gene.

ADMINISTRATIVE REPORT

Personnel changes in LMSF during FY'93 include the recruitment of John Klena (University of Virginia) and Thomas Duensing (Creighton University School of Medicine), Syntex Corporation, Palo Alto, California, as IRTAs; Vladimir Tolstikov (Institute of New Antibiotics, Moscow, Russia) as a Visiting Scientist; and Cheryl Dooley (University of Iowa) as a Pre-IRTA. Summer IRTA students were Dylan Goheen (Princeton), Ralph Liebert (University of Montana) and Jenifer Tekchandani (University of Michigan). Seminars were given by a number of outside guests: Peter Emau (University of Wisconsin), Gary Rathbun (Howard Hughes Medical Institute), Robert Lightowlers (University of Newcastle-upon-Tyne), Zosia Lightowlers (University of Newcastle-upon-Tyne), Adriana Sadziene (University of Texas Health Science Center at San Antonio), Claudia Hase (University of Missouri), Lawrence Loomis (Jackson Foundation for the Advancement of Military Medicine Research Lab), Rebecca Tart (Bowman Gray School of Medicine of Wake Forest University), Michael Wessels (Harvard Medical School), Jos Van Putten (Max-Planck-Institut fur Biologic Infektion), Daniel Dykhuizen (SUNY, Stony Brook), Allen Rogerson (St. Lawrence University), and John McKinney (Rockefeller University).

HONORS AND AWARDS

Awards:

J. Heinemann - ICAAC Young Investigator Award

Journal Editorial Boards:

J. Swanson - Infection and Immunity
S. Pincus - Medical advisory board for Arthritis Today

Manuscripts were reviewed by LMSF staff for the following journals: Canadian Journal of Microbiology, Cell, Infection and Immunity, Journal of Bacteriology, Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of Clinical Microbiology, Journal of Experimental Medicine, Journal of General Microbiology, Journal of Immunology, Journal of Infectious Diseases, Journal of Rheumatology, Microbial Pathogenesis, Molecular Microbiology, New England Journal of Medicine, Proceedings of the National Academy of Sciences USA, and Trends in Microbiology.

Professional Posts:

J. Heinemann - Affiliate Faculty, University of Montana, Missoula, MT

S. Pincus - Adjunct Associate Professor of Internal Medicine, University of Utah, Salt Lake City, UT

Invited Lectures and Participation in Meetings and Symposia:

R. Ankenbauer - Fourth International Symposium on *Pseudomonas*, Vancouver, BC

R. Belland - Eighth International Pathogenic Neisseria Conference, Cuernavaca, Mexico

S. Fischer - Eighth International Pathogenic Neisseria Conference, Cuernavaca, Mexico
Academy of Clinical Laboratory Physicians and Scientists Meeting, New Haven, CT

J. Heinemann - Risk Assessment of Genetically Engineered Organisms, Duluth, MN
American Academy of Microbiology colloquium "Strategies and Mechanisms for Field Research in Environmental Bioremediation," San Antonio, TX

N. Margolis - Department of Biochemistry, Rutgers University, Piscataway, NJ
Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO

S. Pincus - Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, CA
Laboratory of Tumor Cell Biology Annual Meeting, NCI, Bethesda, MD
AIDS Chemotherapy Meeting, London, England
Second International Congress on Biological Response Modifiers, San Diego, CA
University of Manitoba, Winnipeg, Manitoba, Canada
Neutralization of HIV-1 Workshop, Bethesda, MD
New York University, Public Health Research Institute, New York, NY
Chaired the Arthritis Foundation National Research Conference, Phoenix, AZ
Workshop on Cell Expansion, DAIDS, NIAID, NIH, Rockville, MD

P. Rosa - University of Vienna, Vienna, Austria
Czechoslovak Conference on Borreliosis, Prague, Czechoslovakia
Review of the National Surveillance Case Definition for Lyme Disease, CDC, Atlanta, GA
Banbury Conference--Molecular and Immunologic Aspects of Lyme Disease, Cold Spring Harbor Laboratory, NY
Arthritis Foundation National Research Conference, Phoenix, AZ

J. Swanson - Eighth International Pathogenic Neisseria Conference, Cuernavaca, Mexico
First Bristol-Myers Squibb Symposium on Infectious Disease Research, Monterey, CA
Northwest Branch of the American Association for the Advancement of Science STD Symposium, University of Montana, Missoula, MT

K. Tilly - Ribi Immunochem, Hamilton, MT
Keystone Symposium - Bacterial Chromosomes, Keystone, CO
University of Montana, Missoula, MT

V. Tolstikov - Fifth International Conference of the NCDDG-HIV, Washington, DC

Other Activities:

J. Heinemann - Reviewed book for Quarterly Review of Biology

J. Swanson - Reviewed research grants for National Science Foundation, Washington, DC
Consulted for Sexually Transmitted Diseases Branch, NIAID, Bethesda, MD

K. Tilly - Ad hoc reviewer for NSF grant

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00193-14 LMSF

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. Swanson	Chief	LMSF, NIAID
Others:	J. Klena	IRTA Fellow	LMSF, NIAID
	S. G. Morrison	Microbiologist	LMSF, NIAID
	J. M. Wilson	Biologist	LMSF, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	1.5	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies in FY'93 on structure and function of gonococcal surface components were continuations of previously-initiated attempts to describe biological behaviors of these organisms to the physicochemical attributes of their surfaces. Earlier results on electrophoretic characteristics of whole gonococci established that both lipooligosaccharide (LOS) and Opa outer membrane proteins exert individual and collective influences on cell surface charge. Some Opa proteins contribute positive charge on whole gonococci. Other Opa proteins greatly enhanced negativity in spite of their having abundant cationic amino acids in their (predicted) surface-exposed portions. This affect was shown to result from accretion of polyanions (such as DNA and sulfated polysaccharide) on cells that expressed certain Opa proteins. These Opa+ gonococci, in effect, acquire a "garbage" capsule. Most recent studies examined the effects of piliation on gonococcal surface charge and behavior. The results show that pilus+ cells are less negatively charged than their pilus$^-$ counterparts. Piliation 'dampens' influences of LOS and Opa on cell charge, with pilus$^+$ cells maintaining a net charge near zero, often slightly positive, regardless of the environment or which outer membrane components they express. Variant pilus$^+$ organisms that synthesize different pilin subunits differ in both their electrophoretic mobilities and their comparative adherences to eukaryotic cells, but EPM correlates imperfectly with adherence levels. If pilus$^+$ LOSb Opa$^-$ cells are sialylated, their EPM is shifted to very negative values. Similar phenomena accompany sialylation of OpaC cells; when these variants are unsialylated, they bind DNA, but DNA no longer binds when the cells have been sialylated. In contrast OpaA$^+$ variants bind DNA whether or not their LOS is sialylated. These phenomena reinforce other data suggestive that these two Opa proteins are quite differently disposed on the gonococcus' surface.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00516-06 LMSF

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoglobulin Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S. H. Pincus	Medical Officer	LMSF, NIAID
Others:	T. D. Duensing	IRTA Fellow	LMSF, NIAID
	H. Fang	Visiting Fellow	LMSF, NIAID
	V. Tolstikov	Visiting Scientist	LMSF, NIAID
	R. L. Cole	Chemist	LMSF, NIAID
	K. Messer	Bio Lab Tech	LMSF, NIAID

COOPERATING UNITS *(if any)*

Z. Pei, Special Volunteer

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this work is to study the structure and function of the immunoglobulin molecule. The work is being performed so that this understanding will be applied to the development of antibodies for human therapy.

A. Therapeutic systems. 1. Antibodies to surface antigens of group B streptococci (GBS) have been demonstrated to have protective efficacy in a model of neonatal sepsis. We have identified colony opacity variants of GBS and studied their interactions with antibodies and other components of the immune system. 2. The efficacy of anti-HIV antibodies coupled to ricin A-chain has been studied *in vitro*. Monoclonal and polyclonal antibodies directed against different epitopes have been tested. Biological variants of HIV that escape killing with these immunotoxins and CD4-PE40 have been identified. The phenotype of the cells carrying these HIV has been studied, and the molecular mechanisms of immunotoxin escape have been evaluated. Immunotoxins are being tested *in vivo* in well-studied animal systems. 3. Evaluation of the anti-HIV antibody response in subjects exposed to the IIIB/LAV isolate of HIV. Subjects include humans and chimpanzees infected with the virus or immunized with envelope subunits.

B. Antibody Engineering. We are using both genetic and chemical approaches to alter the antibody molecule and then study the effect. Using genetic approaches, we have studied the ability of immune complexes containing chimeric antibodies to interact with antigen and with effector functions. Chemical engineering of antibodies includes the conjugation of anti-HIV drugs to antibodies and the design of cross-linkers that are specifically cleaved by HIV protease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00549-05 LMSF

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Variation and Adaptation in *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	P. A. Rosa	Senior Staff Fellow	LMSF, NIAID
Others:	K. L. Tilly	Senior Staff Fellow	LMSF, NIAID
	N. Margolis	IRTA Fellow	LMSF, NIAID
	D. M. Hogan	Microbiologist	LMSF, NIAID

COOPERATING UNITS *(if any)*

J. Fuhrman, Special Volunteer & Summer Student; J. Tekchandani, Summer Student; A. Sadziene and A. Barbour, University of Texas at San Antonio; and T. Schwan, S. Samuels and J. Hinnebusch, LVP

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our broad objective is to understand the means by which *Borrelia burgdorferi* establishes an infection:transmission cycle between the tick vector and mammalian reservoir host, both of which are needed to maintain the spirochete in nature. The following research efforts relate to this goal.

1. Outer surface protein variation. The outer membrane of *B. burgdorferi* contains several abundant proteins (Osps) that are variable in size and expression and of unknown function. It is likely that the different Osps confer distinct properties on the spirochete that are pertinent to the disparate environments in which it must survive. Rosa and Hogan have identified homologous recombination between *osp* genes in natural populations of *B. burgdorferi* in ticks. Dr. Margolis has also determined that transcriptional regulation or mRNA stability is responsible for different patterns of Osp synthesis among clonal variants. He has cloned an *osp* gene that is variably expressed in *B. burgdorferi* in culture and is investigating the sequences and factors involved in the regulation of its expression.

2. Linear plasmid replication. An understanding of the unusual linear structure of the *Borrelia* chromosome and plasmids is of intrinsic interest and practical merit. Dr. Tilly has isolated homologs of heat shock genes that are involved in plasmid replication in other systems. These genes are induced by a temperature upshift as would be experienced upon transfer from a tick to a mammal. She has isolated and sequenced the gene of the *Borrelia* IHF homolog, another candidate gene that may be involved in linear plasmid replication, and is characterizing its biological activities in *E. coli*. She has identified unique concatemeric or circular forms of the linear plasmids that could represent replication intermediates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00568-04 LMSF

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis and Extent of Microbial Sex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	J. A. Heinemann	Staff Fellow	LMSF, NIAID
Others:	R. G. Ankenbauer	IRTA	LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

My laboratory studies two examples of accelerated genetic changes in microbes. The first is called bacterial conjugation, a process that permits the exchange of genetic information between cells and has the potential to deliver as much as an entire bacterial chromosome. Whereas traits are normally inherited according to Mendelian principles, through passage from parents to offspring, bacteria can exchange genes with neighbors thereby creating hybrid genotypes. Conjugation is mediated by plasmids that can be transferred from many bacterial species to a large range of heterologous species, including eukaryotes. The second, called directed or Cairnsian mutation, may engage a quasi-Lamarckian strategy to accelerate genetic changes when microbes encounter a lethal challenge. Although the molecular details of this mechanism remain to be elucidated, documentation of its existence in bacteria and yeast is accumulating. My laboratory has explored a putative example of Cairnsian adaptation in eukaryotes. We found that the phenomenon was an unexpected and partial adaptation to environmental challenge that could be an intermediate phenotype in the pathway to full adaptation.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00608-03 LMSF

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression and Phase Variation of Gonococci opa Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R. J. Belland	Visiting Associate	LMSF, NIAID
Others:	T. Chen	Visiting Fellow	LMSF, NIAID
	J. Swanson	Chief	LMSF, NIAID
	S. Fischer	Research Associate	LMSF, NIAID
	S. Morrison	Microbiologist	LMSF, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Major constituents of the bacterial outer-membrane of *Neisseria gonorrhoeae*, including the lipooligosaccharide (LOS), pilin, and Opa proteins have been demonstrated to change expression states and antigenic nature during controlled challenge studies in human volunteers. Opa genes exhibit high frequency phase variation (on-off expression) due to changes at the DNA level resulting in translational frame-shifting. Phase variation occurs when a gene undergoes an insertion or deletion of one or more pentameric repeat units (i.e. [CTTCT], referred to as a coding repeat or 'CR') which encode the central portion of the signal-peptide. These changes are independent of homologous recombination pathways and are therefore referred to as 'non-homologous' or 'illegitimate' recombinational (IR) events.

Factors influencing IR in a general manner have been determined in *E. coli* using *opa::pho*A fusions on low copy number vectors. Genes encoding the homologs of the cellular enzymes found to influence phase variation have been identified in *N. gonorrhoeae*, and their role in *opa* phase variation is presently being evaluated by deletion mutagenesis. Transcriptional influences on *opa* phase variation are being investigated by determining the efficiency of transcriptional initiation by each of the three different promoter types found in the *opa* genes of strain MS11. The role of structural elements within the repetitive region of *opa* genes has been extended by the demonstration that intermolecular DNA triplexes form with the addition of the pyrimidine sense repeat oligonucleotide.

Interactions of Opa+ *N. gonorrhoeae* and *E. coli* with PMNs and epithelial cells have shown that expression of specific proteins leads to adherence to both of these cell types. Chimeric versions of Opa fusion proteins do not clearly identify regions of the protein which impart these biological activities. Recombinant Opa fusions have been shown to destabilize the outer-membrane of *E. coli* K12 strains. Strains producing *E. coli* B type LPS and hybrids of K12/B type LPS (e.g. HB101) are better suited to biological assays with Opa fusion proteins. The interaction of Opa+ *N. gonorrhoeae* with normal human sera has been partially characterized. While Opa expression does not appear to directly influence survival in human serum, the addition of human urine does appear to impart an Opa-specific protective effect (which is most pronounced in Opa+, Pili+ bacteria).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00609-03 LMSF

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthetic Gene Approach to Study the Action of Antimicrobial Genes of Mammals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: K. S. Bhat Senior Staff Fellow LMSF, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neutrophil and macrophage cells express several proteins which defend the host from invading bacteria. The main goals of this project are to study the antimicrobial activity of a small group of proteins called defensins and to identify new macrophage specific gene products that might regulate bacterial killing. During the current year, employing cDNA sequencing approach, I have identified a large number of new genes that are expressed in a human macrophage cell line. For this purpose, a high quality cDNA library was constructed using THP-1 cell mRNA. Thus far 75 genes have been isolated. These genes were identified by extensive sequence analysis. All the characterized cDNAs contained an identifiable open reading frame. Among the newly discovered genes are a new superoxide dismutase (SOD) gene. The protein sequence encoded by this new SOD gene shows all the functional motifs of a CuZn SOD enzyme. However, at the DNA level the gene is distinct from the known cytoplasmic SOD. Another newly discovered gene product shows considerable homology to nematode chitinases. Other newly discovered genes include a macrophage specific proteinase inhibitor, tyrosine kinase gene, and a gene coding for a nuclear regulatory protein. A small percentage of the characterized cDNAs shows no homology to any of the known proteins. The primary structure of about 12 genes have been completely determined. This work required extensive DNA sequence analysis. For this purpose, I have constructed a new plasmid vector with features for rapid and simplified subcloning.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00632-02 LMSF

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Siderophores and Iron Transport in *Pseudomona aeruginosa*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R. G. Ankenbauer	IRTA Fellow	NIAID, NIH
Other:	C. A. Dooley	Pre-IRTA Fellow	NIAID, NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.0	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pseudomonas aeruginosa is an important opportunistic pathogen responsible for high mortality in cystic fibrosis and hospital-acquired infections. The damage caused by *Pseudomonas aeruginosa* is highly dependent upon the ability of the organism to obtain iron from the host. If iron is not available to the organism, the bacterium is unable to grow or cause disease. I am concentrating on the study of how this bacterium acquires iron from the host. *P. aeruginosa* produces iron-sequestering compounds called siderophores which help the bacterium get iron from the host or environment. One of these siderophores is called pyochelin and is the subject of my work. The bacterium is able to transport iron into the cell when it is bound to pyochelin. This occurs through a surface protein receptor on the bacterium. The gene for this surface protein has been cloned and sequenced. The receptor for the iron-pyochelin complex is quite similar to receptors for other iron-siderophore complexes which have little structural similarities to the iron-pyochelin complex. This result indicates that many bacteria use very similar systems for the transport of iron into the cell. The similarities of these systems suggest that anti-microbial chemotherapy based on iron-siderophore transport systems may be applicable to a broad range of bacterial infections. An additional finding is that the iron-pyochelin receptor is expressed only under conditions of low iron available. This iron-regulated production of the receptor is caused by a specific DNA sequence called the 'iron box', and this is the first example of such a DNA sequence to be identified in *P. aeruginosa*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00633-02 LMSF

PERIOD COVERED
October 1, 1992, to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neisserial-Host Interaction: Pathogenic Mechanisms and Host Responses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. H. Fischer	Research Associate	LMSF, NIAID

COOPERATING UNITS (if any)
M. Konkel, LVP, RML, NIAID

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interactions of *Neisseria gonorrhoeae* with human neutrophils and serum were studied to delineate possible pathogenic mechanisms. Studies were carried out to (A) characterize PMN non-opsonic recognition of Opa+ gonococci and (B) to try to identify other gonococcal interactions with host components which may be mediated by Opa proteins.

(A) Neutrophil non-opsonic recognition of Opa+ gonococci was investigated. (1) Earlier studies using oligopeptides to test for neutrophil recognition of the Opacity protein fourth loop sequence were expanded to include an additional scrambled control peptide with the identical amino acid composition as the normal sequence peptide. As before, the control peptide showed neither a dose-dependent inhibition of PMN recognition of Opa+ gonococci or direct stimulation of PMNs when coated on surfaces while the normal sequence peptide did both. These findings suggest a role for the Opa protein fourth loop in gonococcal-neutrophil interactions. (2) Monoclonal antibodies against PMN surface proteins have been utilized to begin to characterize PMN surface receptors which may be mediating recognition of Opa+ gonococci. Preliminary results indicate that the PMN CL response to Opa+ gonococci is mediated, at least in part, by the integrin receptor family.

(B) Studies of gonococci exposed to normal human serum have demonstrated that Opa+ gonococci show relatively more serum resistance than Opa- gonococci when the bacteria express the lipooligosaccharide type B phenotype. All gonococci expressing lipooligosaccharide type A phenotype demonstrate an extremely high level of serum resistance, resistant to killing by greater than 75% normal serum.

PHS 6040 (Rev. 5/92)

LABORATORY OF PARASITIC DISEASES
1993 Annual Report
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LABORATORY OF PARASITIC DISEASES

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

The Laboratory of Parasitic Diseases (LPD) has learned that there is still life after loss of its malaria research component to the creation of a separate Laboratory of Malaria Research (LMR). This will entail significant disruption and the trauma of moves for several LPD labs, but we trust that the unhappiness will heal without permanent scars. Sharing of space, equipment, and joint weekly research seminars has preserved a close and mutually beneficial relationship between the two labs which we hope will continue. Further evidence of the joint relationship of the Parasitic Diseases and the new Malaria Laboratory is their representation, along with the Laboratory of Infectious Diseases, as the Intramural Center for Tropical Diseases, with Dr. Neva as Director. This is the new entity, referred to in last year's report, which will link the NIAID Intramural laboratories with the Extramural Programs in the area of parasitology and tropical diseases. So far, the results of this grouping, referred to as the NIAID International Centers for Tropical Disease Research, or ICTDR, have been two annual meetings, the second one being held in Bethesda April 28-30, 1993, with the major emphasis being presentations upon vaccines, vaccine development and applications, and new technologies for diagnostics. Drs. Alan Sher of LPD (cytokine pathways of immune response), Tom Wellems of LMR (Dx of malarial drug resistance) and Al Kapikian of LID (rotavirus vaccines) made presentations on behalf of the Intramural Labs.

There is concern over a number of departures at high level positions that are imminent in the administrative staff of the Intramural program. Although it is sometimes difficult for the research staff to understand and to be fully convinced, the increase in administrative activities in the DIR Director's office over the last decade, which inevitably spills over to involve scientific staff, presumably reflects demands from Building 1 that cannot be avoided.

Two long-time members of the Section on Biochemistry and Physiology, Eugene Weinbach, Ph.D. (Section Head), and Teresa Mercado, Ph.D., retired in January, 1993. We will miss Gene Weinbach's annual discourse on electron transfer and the mysteries of mitochondria during LPD seminars, and Teresa's tenacious quest to elucidate the nature of the anti-trypanosomal factor elaborated by species of *Pseudomonas*. Other departures included Andrea Cooper, Ph.D., from the U.K., who accepted a position at Colorado State University to work on immunology of experimental tuberculosis. T. Nozaki, M.D., the Visiting Fellow from Japan, went to Rockefeller University with Dr. George Cross on another postdoctoral fellowship. The only professional to join LPD in the past fiscal year, as of this writing, was Steven Mawhorter, M.D., who comes from an infectious disease fellowship at Case Western to work on eosinophils. However, a number of new research fellows will be joining LPD late in the fiscal year. Dr. Zilton Andrade from Brazil came (with his wife, Sonia, also a pathologist, who will work in the Heart Institute) for his last few months as a Fogarty Scholar.

The second annual Gorgas Memorial/Leon Jacobs endowed lectureship, made possible by a gift to LPD from the Gorgas Memorial Foundation, was given by Professor Elmer Pfefferkorn of Dartmouth University in November. He spoke about his research on *Toxoplasma*.

Sometime during the next year we hope to have available a followup of what has happened to research fellows who came to LPD for at least one year of research training during the period of 1970 to 1990. A survey has been mailed out to about 140 trainees, with a high percentage of response. It will be interesting to learn how many of the LPD trainees are still engaged in research.

Travel abroad to conduct field research by LPD personnel was substantial, as usual. Drs. Sacks and Kenney spent 1 to 2 weeks in India in efforts to restart our studies on visceral leishmaniasis in Patna. Drs. Tom Wynn and Megan Williams were in Recife, Brazil, to work with Brazilian colleagues on cell-mediated and cytokine responses in patients with *S. mansoni* schistosomiasis. Drs. Ottesen and Zimmerman traveled to Madras, India, to collect cells from a spectrum of lymphatic filariasis patients, which will be used in a new molecular approach to examine a possible genetic basis for clinical outcome of filarial infection. A similar approach will be used to explore whether linkages of the Class II major histocompatibility complex (MHC) play a role in determining disease caused by *Onchocerca volvulus* in two different racial groups in Ecuador. Drs. Nutman, Elson and Zimmerman were involved in this project with their Ecuadorian collaborators. Dr. Kenney went to Brazil to initiate a study in collaboration with Dr. Reynaldo Dietze on genetic susceptibility to leishmanial infection. Dr. Neva traveled to Honduras to explore the initiation of a double-blind topical treatment trial of cutaneous leishmaniasis.

HONORS AND AWARDS

All of the permanent professional staff, and many of the senior nontenured staff serve on Editorial Boards of scientific journals and/or frequently review manuscripts for journals. Such activities reflect the high standing of LPD scientists by professional peers. Also many of the staff serve on Ad Hoc Committees for granting agencies or foundations, and are invited to participate in workshops and scientific meetings. Such activities are generally not cited here individually unless felt to deserve special mention.

During the past year Dr. Allen Cheever was a recipient of the Meritorious Service Medal of the U.S.P.H.S. Commissioned Corps, and Dr. Thomas Nutman was elected to membership in the American Society for Clinical Investigation. Dr. Alan Sher accepted an appointment as member of the Scientific Advisory Board of the International Laboratory for Research on Animal Diseases (ILRAD) in Nairobi, Kenya.

Clinical Parasitology Section

Immunoregulation in parasitic infections. Eosinophilia is one of the hallmarks of parasitic infections caused by helminths. Of the several cytokines that have been implicated in eosinophilopoesis (IL-5, GMCSF, IL-3), neither IL-3 or GMCSF seems to be involved in the eosinophilia seen in parasitic infections. On the basis of *in vitro* (induction of IL-5 by parasite antigens in lymphocytes of patients), as well as *in vivo* data (IL-5 levels in serum precede rise in eosinophils), IL-5 is clearly the cytokine mediating the eosinophilia of parasitic infections. However, IL-2 is needed to induce IL-5 production (Nutman and Mahanty). The B cell response characteristically seen in helminth infections is an increase in serum IgE. This is also associated with an increase in the level of production of antibody in the subclass IgG4. Interestingly, other IgG subclasses (of 1, 2 and 3) are not involved. Again, as with the IL-5 and the eosinophilia relationship, *in vitro* and *in vivo* experiments indicated that the IgE and IgG4 responses are dependent upon the cytokine IL-4. In contrast, INF-γ tends to inhibit the IgG4 and IgE responses (Mollis and Nutman). The type of T lymphocyte response characterized by production of IL-4 and IL-5 has been referred to as a TH2-type response. Some progress has been made in identifying the lymphocytes responsible for TH2 responses. By use of flow cytometry and reagents that identify cell surface markers, it was shown that 100-fold enrichment for TH2 type cells could be obtained by selecting CD4+, CD45RO+ and CD 27 negative cells (Elson, Shaw of NCI, and Nutman).

Immunopathology vs. immune tolerance resulting from filarial infections. Filarial infections are known to result in a spectrum of clinical manifestations, ranging from asymptomatic microfilaremia (MF) to hyper-responsive states such as tropical pulmonary eosinophilia. The immunologic basis for the immune tolerance in a group of microfilaremic (MF) patients without symptoms was shown to be mediated by the anti-proliferative cytokines IL-10 and TGF-β. Such activity was not found in a group of patients with pathology due to lymphatic filariasis. In addition, the MF patients showed a predominant TH2-type response when their cytokine-secreting peripheral lymphocytes were examined. Furthermore, neutralization of the IL-10 or TGF-β significantly increased lymphocyte proliferation to filarial antigens in the MF group of patients (Mahanty, Ottesen and Nutman).

Another example of factors affecting immune responsiveness to filarial infection is seen in prenatal exposure to maternal filariasis. In a 17-year follow up study in the Cook Islands, children born to infection-free mothers had significantly greater lymphocyte proliferative responses and produced higher levels of IL-2, IL-5 and GMCSF in response to filarial antigens than did children born to microfilaremic mothers. This suggests prenatal "tolerization," and may have significant implications as to whether there will be subsequent development of disease (Steel, Ottesen, Zimmerman).

However, the apparently normal microfilaremic person does not always escape pathology. The late follow-up visit to the filariasis endemic Cook Islands disclosed that almost 50 per cent of this group had hematuria and/or proteinuria. Studies of similar patients elsewhere indicate that the presence of lymphatic system abnormalities by ultrasound and lymphoscintigraphy is rather common (Ottesen and McCarthy).

New findings have linked the cytokines IL-6 and TNFα as important immunologic mediators in the Mazzotti reaction, a post-treatment inflammatory response seen after treatment of onchocerciasis and filariasis patients with diethylcarbamazine (Ottesen).

Populations in filarial endemic areas provide clues to protective antigens. As reported last year, the concept of screening the sera of apparently immune individuals within an endemic area by Western blot for protective antigens was continued. A 43kD larval antigen was identified which made it possible to isolate a recombinant antigen from a *W. bancrofti* genomic DNA library. The sequence of this antigen is that of a filarial chitinase, an enzyme not found in mammalian hosts, and a potential vaccine candidate (Raghavan, Nutman and Ottesen).

Detection of parasite DNA in diagnosis of filarial infections by PCR is a logical application of molecular biology. Amplification of parasite DNA in skin snips, which is an accepted technique to demonstrate microfilariae of *Onchocerca*, is being tested from specimens collected in the field. Some specimens negative by microscopy have been PCR-positive, but final results are not yet in (Nutman, Zimmerman and Elson). The same approach is being tried with blood samples from patients with lymphatic filariasis (McCarthy and Williams).

Genetic susceptibility to filarial infection by analysis of MHC Class II linkage. The human leukocyte antigens (HLA) that serve as the basis for tissue typing consist of two functionally different classes of glycoproteins, referred to as major histocompatibility complexes (MHC) I and II. The MHC Class II proteins react with CD4 T cells in antigen recognition. Increasingly, PCR technology is replacing traditional serologic tissue-typing to distinguish MHC II alleles. A simplified PCR technique, using only 3 DNA probes, called heteroduplex analysis, is being used for genotyping DQA and DQB alleles. This approach to determine MHC Class II linkages is being applied to two different groups of filariasis patients in an effort to determine if clinical outcome is controlled by genetic factors. Both groups of patients are unique in that they are very well characterized clinically. One is the Mauke Island population in the Cook Islands on whom a 17-year follow-up observation period was available, and who exhibit a range of clinical manifestations of lymphatic filariasis (Zimmerman, Ottesen and Williams). The other group is in Madras, India, which includes an even broader range of filarial disease outcomes (Zimmerman, Ottesen and Nutman).

Treatment of filarial infection and disease. Dr. Ottesen of LPD is a member of a W.H.O. sponsored group evaluating the use of ivermectin in the treatment of lymphatic filariasis. The drug is being used for several purposes: (1) high doses at intervals as a macrofilaricide (kill adult worms), (2) monthly doses to reduce incidence of inflammatory manifestations, (3) repeated mass administration to reduce microfilaremia and control transmission, and (4) combined with DEC as a macrofilaricide. Repeated one-week courses of DEC at monthly intervals is being tested in the Cook Islands as a macrofilaricide (McCarthy and Ottesen).

The efficacy of DEC in treatment of loiasis in expatriates, which was initially thought to be very high, is being re-evaluated because of the >100 patients seen at NIH only about 50 per cent responded to a single 3-week course of high dose (8-10mg/Kg) DEC. Up to 10-20 percent of those who have received 3 to 4 such courses continue to have symptoms (Nutman and Ottesen). Because severe adverse reactions to DEC may occur in loiasis patients with microfilaremia, alternative treatment with albendazole was tried with patients in Benin, West Africa. In 23 microfilaremic patients, microfilarial levels did not decrease until 2 weeks after starting albendazole, with 80 percent clearance at 6 months after treatment. This suggests an effect on adult worms (Nutman).

Diagnosis and treatment of strongyloidiasis. One of the recombinant products expressed by a clone from a cDNA library from *S. stercoralis* filariform larvae from λgt11 was reactive with IgG from all 8 patients and IgE in all but 1 of the 8 patients infected with the parasite. A partial sequence of a PCR product of a clone showed homology to a collagen gene present in *C. elegans* and *B. malayi*. We plan to screen the cDNA library for more clones and prepare a genomic DNA library with the objective of developing recombinant products for diagnostic use (Gam, Raghavan and Neva). The controlled trial comparing thiabendazole with Ivermectin in 53 Cambodian immigrants in Lowell, Mass., who were infected with *S. stercoralis,* showed >90 percent efficacy with both drugs; there was one treatment failure in 18 cases treated with a three-day course of thiabendazole, 1 failure in 18 cases treated with a single 200 micrograms/kg dose of ivermectin, and no failures in 17 patients who received the same ivermectin dose given in two successive days. There were many of the usual side effects from thiabendazole and very few with ivermectin. Post-treatment antibody levels remain to be tested (P. Gann of Northwestern Univ., Neva, and Gam).

Immunology and Cell Biology Section (Schistosomes and Toxoplasma)

Regulation of schistosome egg granulomas by cytokines. Last year it was reported that the cytokine IL-4, representing a Th2 response, played a critical role in formation of schistosome egg granulomas. Since Th2 responses are known to be down-regulated by INF-γ, measures to manipulate the activity of INF-γ were tested for their effect on egg granulomas. When monoclonal antibody (Mab) to INF-γ was used to treat mice that had been injected intravenously with schistosome eggs to produce pulmonary granulomas, it resulted in a dramatic enhancement of granuloma size. A similar result of increased granuloma size was seen in mice injected with anti-natural killer (NK) cell antibodies, suggesting that NK cells are the source of INF-γ. This concept was supported by the finding that IL-12, a cytokine that stimulates INF-γ production by NK cells, suppresses granuloma formation in the lungs of injected mice, as well as in livers of naturally infected mice. These results raise the possibility that certain types of pathology might be treated by administration of IL-12 to induce INF-γ production from NK cells (Wynn, Oswald, and Eltoum).

Cytokine responses in *S. mansoni*-infected patients. In cooperation with Brazilian collaborators in Recife, Brazil, cytokine responses of peripheral blood mononuclear cells of patients with *S. mansoni* infections were examined. Elevated IL-4 and IL-5 responses to egg and worm antigens, consistent with Th2-type reactions were seen. Interestingly, cytokine responses to mitogens (instead of schistosome antigens) with cells of patients were of the Th1 variety, in contrast to the situation in mice (Williams, Wynn and Montenegro and Coutinho of Recife).

Further events in immune mediation during murine toxoplasmic encephalitis. While previous work concerned the role of INFγ in the murine model of toxoplasmic encephalitis, more recent studies have focused upon expression of mRNAs encoding cytokines, as well as macrophage markers. In early stages of infection, up to 2 to 4 weeks, an increase in Th1 cytokine mRNAs (INF-γ and IL-2) was found, as well as expression of IL-1, IL-6, IL-10, GMCSF and INF-α. However, after 8 weeks of infection with *T. gondii*, a dramatic decrease in Th1 cytokine and most monokine mRNAs was observed. These changes are interpreted as evidence that reactivation of *T. gondii* results from down-regulation of INF-γ and TNF-α expression (Gazzinelli). A special type of NK cell (NK1.1+) produced in the spleen was found to be a source of INF-γ and protection against toxoplasmosis in MHC Class I deficient mice which also fail to produce CD8+ lymphocytes. This provides additional evidence for the importance of INFγ in controlling *T. gondii* infection *in vivo*. These experiments also demonstrate that a defective host immune system can sometimes utilize an alternate pathway to produce protective INF-γ (Denkers, Gazzinelli and Sher).

Role of Il-12 in the induction by *T. gondii* of INF-γ from NK cells and the use of the cytokine in the stimulation of resistance. One mechanism of production of INF-γ by NK cells of *Toxoplasma*-infected mice described last year requires adherent accessory cells and TNF-α. Now, a newly characterized cytokine, IL-12, was found to be necessary to induce INF-γ by NK cells in *Toxoplasma*-infected mice. Anti-IL-12 antibodies completely inhibited *T. gondii* or endotoxin-stimulated INF-γ production by NK-enriched spleen cells from SCID mice. Treatment of toxoplasma-infected SCID mice with recombinant IL-12 resulted in prolonged survival by means of a response dependent upon both INF-γ and NK cells. Thus, it appears that IL-12 is required for the T-independent triggering of NK cells by *T. gondii*, and that this cytokine may be important in inducing this protective pathway in immunodeficient hosts. (Gazzinelli, Hieny, Wynn and Sher).

Surface membrane protein s of *Toxoplasma gondii*. A 5'-nucleotidase (5'-NT) in surface membranes of *T. gondii* tachyzoites has been identified and characterized. This enzyme cleaves 5' nucleotides of which 5' uridine monophosphate is the preferred substrate. This fits with the observation that the parasite rapidly takes up uracil from the host cell. Oligonucleotides, reflecting sequence homologies of the few 5'-NTases which have been cloned to date, are being used as primers for PCR with genomic and cDNAs of *T. gondii*. The PCR products are being used as probes to identify the parasite 5'-NT gene in Southern and Northern blots (Russell and Dwyer).

Immunology and Cell Biology Section (*Leishmania*)

Molecular characterization of leishmanial 3' nucleotidase (3'-NT) and secretory acid phosphatase (SAcP) genes. After purification of 3'-NT from surface membranes of *L. donovani* promastigotes, 4 peptides were obtained for N-terminal amino acid sequencing. Using synthetic oligonucleotides for these sequences as primers in PCR reactions with parasite genomic DNA, a 294 base pair PCR product was obtained which hybridized with high specificity to genomic DNA in Southern blots. The PCR product is being used to screen both genomic and cDNA libraries for the complete 3'-NT gene (Debrabant and Dwyer).

About one third of the 5' end of the gene encoding the *L. donovani* SAcP has been sequenced from a cloned fragment of genomic DNA. Southern blot analyses indicate that there are at least 3 different copies of the SAcP gene, and they are not tandemly arrayed in the genome. Several cosmid clones have been made for restriction-site mapping and nucleotide sequencing (McCarthy-Burke and Dwyer).

By using monoclonal antibodies vs. leishmanial SAcP, the production of the enzyme by intracellular parasites, and its movement within the cell can be followed. The SAcP moves out of the parasitophorous vacuole and by 96 hours is disbursed throughout the cell cytoplasm (Harris and Kaye of the London School with Dwyer).

Comparison of surface membrane enzymes of leishmanial promastigotes with *in vitro*-grown amastigotes. Surface membrane activity of 3'-nucleotidase, 5' nucleotidase, and acid phosphatase were equivalent in both morphologic forms of *L. donovani*. Amastigotes were found to have a 120 kDa protease that was not present in promastigotes; conversely, promastigotes were found to express a soluble nuclease activity which was absent in amastigotes (Dwyer, Alleman and Debrabant). The system for growing *L. donovani* amastigotes without cells (elevated temperature and increased serum concentration) has now been extended to virtually all species except *L. braziliensis* (Dwyer). With the objective of identifying genes involved in development and differentiation of the leishmanial parasite, clones were isolated from both promastigote and amastigote cDNA libraries for analysis by subtractive hybridization. Several amastigote-specific clones have been found which code for proteins which have different patterns of recognition by patient's sera (*Kala azar* cases) according to geographic area (Dwyer with collaborators Joshi, Nakhasi and Pogue).

Cell-mediated responses in human leishmanial infections. The cytokine mRNA responses in bone marrow tissue during visceral disease that were reported last year from the Sudan are being studied further in India. The objective, in addition to confirming the elevated levels of IL-10 mRNA found during active disease in cases from the Sudan, is to determine the cellular source of this cytokine by positive and negative selection of different cell types (Kenney, Sacks and collaborators in Patna, India).

Delayed hypersensitivity skin tests, some with experimental antigens provided by the W.H.O., and *in vitro* assays of lymphocyte proliferation to leishmanial antigens were carried out on more "Desert Storm" returnees from the Persian Gulf. Neither type of test was found to be a reliable indicator of recent subclinical visceral infection (Neva and Magill of WRAMC and Ohl of Naval Medical Center).

Developmental biology of *Leishmania*. The story of changes in the lipophosphoglycan (LPG) surface molecule of *Leishmania*, specifically in the type and position of terminal saccharide residues, has not yet been defined for all species of parasite. It was initially worked out for *L. major*, and then extended to *L. amazonensis* and *L. donovani*. Also, the relationship of the different species of sandfly vectors to the LPG structure of the various species of *Leishmania* is an ongoing study (Sacks, Pimenta, Saraiva, Turco of Kentucky and Rowton of WRAIR).

A project has been initiated to investigate the genetic basis of leishmanial metacyclogenesis, i.e., the process by which the parasite becomes an infective form. This is being done by transfection in collaboration with Steve Beverley of Harvard, using his cosmid vector and genomic library of *L. major*. In the process of screening parasite clones for this project it was found that a morphological change in the appearance of colonies occurred as they matured on agar plate cultures, which correlated with stages of development. If this finding can be consistently reproduced, it will greatly facilitate the transfection studies (Kenney and Sacks).

Host Parasite Relations Section

Mouse model for *Giardia* infection. Infection of newborn mice and gerbils with *Giardia lamblia* from humans has been described, but various drawbacks make these models unsuitable for systematic studies. One of nine human *Giardia* isolates tested was found capable of infecting several in-bred strains of adult mice. A persistent low level infection occurred in C3H and BALB/c, but spontaneous cure after the first several weeks was the pattern in DBA mice. SCID mice were unable to suppress infection, and, in contrast to normal mice, continued to express the same variable surface protein. These findings now provide the basis for various immunologic studies in the mouse (Byrd and Nash).

Molecular basis for typing or identification of *Giardia*. Isolates of *Giardia* can be put into one of 3 or 4 groups in the basis of ability to express certain variant specific proteins (VSPs). Because wild and/or domestic animals may be reservoirs of the parasite, identification of the source of the parasite can be a useful epidemiological tool. By selection of appropriate primers, PCR products can amplify variable surface proteins (VSPs) as to group. This technique will be applied to identify *Giardia* in stools. Another gene (GLORF-C4) has been found in *Giardia* of Group 3, and may become useful for identification of parasite strains (Nash and Mowatt).

Biochemistry of *Giardia*. The Zn binding by VSPs of *Giardia* reported last year have been studied further. The motif of two amino acids between cysteine was thought to be the metal-binding region, but fusion proteins which excluded this area also bound Zn. Cysteine was found essential for survival and growth of the parasite, which cannot synthesize the amino acid. Insulin-like growth factor II (IGFII), a component of Cohn fraction IV-1 of blood proteins, was found able to promote growth of *Giardia* in the absence of serum. IGF II also supported uptake of cysteine by the parasite. However, exploitation of this finding in *in vivo* metabolic studies is complicated by the fact that IGFs are present in the intestinal tract of animals (Nash, Lujan, and Mowatt).

Experimental pathology of schistosomiasis. Last year it was reported that anti-IL-4 treatment of mice infected with either *S. mansoni* or *S. japonicum*, if started before egg laying, resulted in a striking decrease in hepatic fibrosis, but very little effect on size of egg granulomas in the liver. However, in current work pulmonary granulomas in mice injected intravenously with eggs were greatly reduced in size in spite of anti-IL-4 treatment. Thus, it appears that patterns of cytokine expression may be organ specific. In other experiments, CD8+ and IL-10 "knockout mice" showed little or no alteration in pathology of *S. mansoni* infection (Cheever, Sher, and Wynn).

Fecundity of experimental schistosome infections. Treatment of mice with anti-TNF-α only slightly decreased numbers of eggs laid by *S. mansoni* females and did not affect egg production by *S. japonicum*. What appeared to be early decreased egg laying by scid mice infected with *S. japonicum* turned out to be a delayed onset in egg laying (Cheever). A reduction in fecundity of *S. mansoni* worms after vaccination with glutathione-S-transferase reported recently by others could not be confirmed. However, there was some question as to adequate immune response (Cheever and Actor).

Parasite Growth and Differentiation Section

Molecular typing of strains of *E. histolyticas*. Now that *Entamoeba histolytica* has been shown to consist of two morphologically identical but distinct species, separable by molecular characteristics and pathogenicity, the next question is how to distinguish between different isolates of the pathogenic variety? Although isoenzyme patterns permit some differentiation, each pathogenic strain does not have a distinctive pattern. If individual strains of pathogenic *E. histolytica* could be identified, it would provide answers to important epidemiological and clinical questions. How many different strains are circulating in a given population? Are certain strains more likely than others to cause liver abscess? By analysis of the polymorphism of two *E. histolytica* genes, isolates collected over the years from widely different geographic areas of the world were found to fall into 16 different patterns. Both genes are amplified by PCR; the serine rich antigen gene (SREHP) is then cut with a restriction enzyme to yield fragments of different sizes, while the strain-specific gene (SSG) is checked for size of the major product. This gene pattern of various strains was found to be stable on (1) long-term *in vitro* cultivation, (2) axenization, (3) cloning, and (4) animal passage. While additional markers for pathogenic strains of *E. histolytica* will undoubtedly be found, these results provide an encouraging basis for future clinical and epidemiological studies of amebiasis (Clark and Diamond).

Incidentally, the isolate identification method provided further evidence that "conversion" of nonpathogenic to pathogenic strains of *E. histolytica* was very likely because of cross-contamination of cultures. In each of the three published accounts of this "conversion," the converted pathogenic strain was found to be the same as the reference pathogenic strain being used in that lab (Clark and Diamond).

More evidence for some type of mitochondrion in *E. histolytica*. A second mitochondrion gene has been demonstrated in *E. histolytica*. It is the same as caperonin, or heat shock protein 60. The fact that the molecule is very immunogenic should allow reagents to be developed for localization of the protein, and for purification of the organelle (Clark and Diamond).

Physiology and Biochemistry Section

Synchronization of DNA synthetic cycle of *Kinetoplastidae*. Cultures of hemoflagellates exposed to appropriate concentrations of hydroxyurea are arrested in G_1 phase of the DNA synthetic cycle. Following removal of hydroxyurea, the G_1 arrested cells resume synthesis of DNAs. Gradually, the population of synchronized cells undergo DNA synthesis (S phase) and complete the DNA synthetic cycle. Synchrony lasts for only one DNA synthetic cycle. Several species of trypanosomatids (*T. cruzi*, *C. luciliae*, and *L. donovani*) have been successfully synchronized and assayed by flow cytometry. Hydroxyurea exposure time and concentration are critical and vary with the species of organism; for *T. cruzi*, concentrations ranging from 2.0 to 20 mM are optimum (Dvorak, McDaniel and Galanti of Chile).

In a somewhat related effort, attempts have been initiated to examine the DNA synthetic cycle of amebae, especially *Entamoeba* species, by flow cytometry. Preliminary results indicate that DNA-specific fluorochromes can be used if the amebae are first fixed with a combination of detergents which permeabilize the organism and remove naturally fluorescing materials (Dvorak, Kobayashi and Diamond).

Cytoplasmic DNA particles in the *Kinetoplastidae*. Study of the nonnuclear DNA particles seen in the cytoplasm of strains of *T. cruzi* by low-light level microscopy wih DNA specific fluorochromes has been continued and extended to other strains of *T. cruzi* and to *Leishmania*. All isolates examined contain these cytoplasmic elements, although there are marked differences in number and size of the particles per cell as well as percentage of cells affected. A technique of fluorescent *in situ* hybridization, or FISH, was developed that uses molecular probes for sequences of either kinetoplast DNA (kDNA) or nuclear DNA (nDNA), which are then visualized with biotin-avidin reagents. Using such a kDNA probe, the kDNA and cytoplasmic DNA particles both hybridized, but the nDNA did not hybridize. The cytoplasmic DNA elements did not hybridize with a nDNA probe. These results indicate that the cytoplasmic DNA is of kinetoplast origin, likely minicircle DNA. Other evidence supporting the mitochondrial nature of the cytoplasmic DNA was the presence of a mitochondrial protein, mtp 70 (Miyahara and Dvorak, Dwyer).

Multivariant analysis of *T. cruzi* stocks. A collaborative project has been initiated with investigators in Argentina and Chile to study the diversity of nuclear histones, shed acute phase antigens (SAPA), the protease, and random amplified polymorphic DNA (RAPD) in various stocks of *T. cruzi* parasites (Cazzulo and Frasch of Argentina, Galanti and Solari of Chile, and Dvorak).

Studies on the anti-trypanosomal factor from *Pseudomonas*. Although Dr. Mercado retired from NIH in January, 1993, she has continued to work on the anti-trypanosomal factor extracted from *Pseudomonas fluorescens*. She continues to collaborate with both NIH and non-NIH investigators on the project (Mercado, Fales and Ferrans of NIH, Hammer of Georgetown, Alssoy of Yale).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZOI AI 00094-34 LPD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Entamoeba histolytica: Cellular physiology and molecular diagnostics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	L.S. Diamond	Section Head	LPD, NIAID
Others:	C.G. Clark	Staff Fellow	LPD, NIAID
	D. Mirelman	Fogarty Scholar	FIC

COOPERATING UNITS *(if any)*
National Zoological Park (Ann Bratthauer)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Parasite Growth and Differentiation

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Entamoeba histolytica has been separated into two species on the basis of molecular evidence obtained through 'riboprinting,' a technique devised in this laboratory, and on the basis of biochemical and immunological evidence obtained elsewhere. The name *E. histolytica* Schaudinn, 1903 has been retained for the invasive species, and *E. dispar* Brumpt, 1925 has been assigned to the noninvasive one.

The first method to positively identify individual isolates of *E. histolytica* has been devised. The technology involved is based on the discovery of extensive polymorphism in two of the parasite's genes, one encoding a surface antigen, the other of unknown function. The method permits addressing important questions such as: Is there more than one strain circulating in a population? Are some strains more accountable than others for invasive disease? Do individual strains have tropisms for specific organs?

The isolate identification method has provided evidence that in each of three published accounts of 'conversions' of *E. dispar* to *E. histolytica*, the 'conversions' resulted from cross-contamination of the *E. dispar* cultures with reference strains of *E. histolytica* present in the laboratories at the time those experiments were conducted.

A second mitochondrion-specific gene has been identified in *E. histolytica*. This implies that other components of the protein importation apparatus will also be present. The existence of this organelle, possibly vestigial, has significant implications for the metabolism of drugs used to treat the infection as well as offering the potential for rational drug design.

Taxonomic studies of the genus *Entamoeba* indicate profound divergence within the genus between species that develop cysts with different numbers of nuclei.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00097-35 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Anti-trypanosomal Factor from *Pseudomonas fluorescens*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. I. Mercado	Research Physiologist	LPD, NIAID
Others:	H. M. Fales	Chief, Lab. of Chemistry	IR, CH, NHLBI
	V. J. Ferrans	Chief, Ultrastructure Section	IR, PA, NHLBI

COOPERATING UNITS *(if any)*

Millipore-Waters Life Science Lab, Fairfax, Va. (J. Stone); Department of Chemistry, Georgetown University, Washington, D.C. (G. Hammer); MacArthur Center for Molecular Parasitology, Yale University School of Medicine, New Haven, CT (S. Aksoy).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0		0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As reported in the annual report for 1991-92, I retired on January 2, 1993. Because of the renovation of the Twinbrook II Facility, I had to vacate my lab space and there was no space available in Building 4 to continue this work. During my retirement, however, I have been able to continue my collaboration with Dr. George Hammer, Department of Chemistry, Georgetown University, the NMR expert on this project. He has identified the two main components of the anti-trypanosomal factor (ATF), a peptide and an α-d-glycoside. There is a third component, which remains to be identified. In order to facilitate the work in the elucidation of the chemical structure, I have obtained the collaboration of Dr. Frank Robey, Chief, Peptide and Immunochemistry Unit, Laboratory of Cellular Development and Oncology, NIDR. We are employing reversed-phase high performance chromatography (HPLC) in an attempt to obtain a better separation of the components for the NMR identification. I have been able to perform the trypanosome bioassays (*Trypanosoma equiperdum*) in space provided in Building 7 by Dr. Meta Timmons, Chief, Animal Facility, Animal Care Branch, NIAID.

An additional, collaborative study is being continued with Dr. Serap Alssoy, MacArthur Center for Molecular Parasitology, Yale University School of Medicine, New Haven, CT. The goal of the study is to clone and express the gene encoding for the AFT-II employing symbionts normally present in the gut of the tsetse fly, the vector of African trypanosomiasis. Re-introduction of such an ATF-producing symbiont into tsetse flies should theoretically prevent trypanosomal infections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00098-37 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.C. Weinbach	Section Head	LPD, NIAID
Others:	L. Levenbook	Research Chemist	LPB, NIDDK
	L. Diamond	Research Zoologist	LPD, NIAID
	M. Mowatt	Senior Staff Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.58	33	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dr. Weinbach retired January 31st, 1993.

Studies on the biochemical and genetic characteristics of a key enzyme of carbohydrate metabolism, triosephosphate isomerase (TIM), in trophozoites of *Giardia lamblia*, were continued this year. In collaboration with Dr. Levenbook, purification of the enzyme was undertaken. Ammonium sulfate fractionations followed by chromatographic separation of the enzyme from other soluble proteins resulted in a 100-fold enrichment of TIM activity. Chromatography revealed that the parasite enzyme has properties which differ markedly from mammalian and trypanosome counterparts.

In collaboration with Dr. Mowatt, transfection experiments with the TIM gene from *G. lamblia* and a TIM chromosomal deletion mutant of *Escherichia coli* showed that TIM activity was now expressed by the mutant bacterium. Complementation with TIM genes suggests the general applicability of the method to cloning other genes for which *E. coli* mutants are available.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00099-23 LPD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
Biophysical Parasitology

PRINCIPAL INVESTIGATOR	J. A. Dvorak	Res. Microbiologist	LPD, NIAID
Others:	J. P. McDaniel	Biologist	LPD, NIAID
	Y. Miyahira	Fogarty Fellow	LPD, NIAID
	S. Kobayashi	Visiting Scientist	Keio Univ., Japan
	C. P. Mudd	Senior Engineer	ACE, BEIP
	H. Casio	Engineer	RR, BEIP
(See below)	J. J. Cazzulo	Head, Para. Biochem.	Fund. Campomar, Argentina

COOPERATING UNITS *(if any)*
Biomedical Engineering and Instrumentation Program, DRR; Research Resources Branch, DRR; Laboratory of Malaria Research, NIAID

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Others: C. Frasch, Head, Mol. Biol., Fund. Campomar, Argentina; N. Galanti, Head, Mol. Biol. Univ. Chile, Santiago, Chile; A. Solari, Head, Mol. Para. Univ. Chile, Santiago, Chile; G. Ward, Senior Staff Fellow, LMR, NIAID; D. Dwyer, Res. Biologist, LPD, NIAID; M. Aiyakawa, Scientist, Case Western; L. Diamond, Scientist, NIAID

This project is concerned with analyses of the genetic diversity of medically important parasitic protozoa and its implications to the epidemiology, course, and diagnosis of disease. The project has become increasingly involved in isolation of diversity at the DNA level. We have developed a method to synchronize the DNA synthetic cycle of trypanosomatids using very high levels of hydroxyurea. We are utilizing the technique for the production of large quantities of synchronized cells for elucidation and analyses of cell cycle-specific and developmental stage-specific substances. We have recently identified the presence in kinetoplastids of cytoplasmic DNA elements which contain kDNA minicircles and mitochondrial proteins. We are utilizing topoisomerase inhibitors to further analyze these DNA particles. It is possible that these particles represent the sites of guide RNA synthesis in the kinetoplastids. We have developed the technology to analyze the DNA synthetic cycle of amoeba by flow cytometry. The technique overcomes the problem of nonspecific background fluorescence which exists when amoeba are prepared for flow cytometry by conventional methods. The low-light-level video microscopy techniques developed for the study of the interaction of malaria merozoites with erythrocytes has been expanded to include the interaction of *Trypanosoma cruzi* and *Toxoplasma gondii* with vertebrate cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00102-19 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. A. Neva	Chief	LPD,NIAID
Others:	D. Sacks	Research Microbio.	LPD,NIAID
	A. Gam	Bio. Lab. Tech. (Micro.)	LPD,NIAID
	R. Kenney	Senior Research Investigator	LPD,NIAID
	C. Karp	NRSA Fellow	LPD,NIAID

COOPERATING UNITS *(if any)*

Walter Reed Army Med. Center (C. Oster & A. Magill); National Naval Med Center (C. Ohl); Ministry of Health, Honduras (C. Ponce); Rajendra Memorial Research Institute, Patna, India (Prabakhar); Dept. Biology, Youngstown State Univ. (R. Kreutzer).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses upon the different clinical forms of leishmanial infections in humans, immune responses to the parasite, with particular reference to cell-mediated responses and the cytokine regulation of these responses, and characteristics of the causative parasites.

Of the 12 patients referred to NIH and cultured for leishmania, only 4 were positive and treated. No explanation could be found for the striking increase in serum lipase and amylase that occurred in association with pentavalent antimony treatment. Tests of cell-mediated immune function (delayed hypersensitivity skin tests and lymphocyte proliferation assays) were carried out in a total of more than 50 Persian Gulf military returnees. No completely satisfactory method of retrospective immunologic diagnosis of leishmanial infection was found for this group of patients, although a number of suggestive cases were identified.

Further semi-quantitative assays for mRNA of various cytokines in the bone marrow of visceral leishmaniasis patients are planned. A controlled clinical trial of topical therapy for cutaneous leishmaniasis in Honduras is under evaluation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00161-16 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR	T.E. Nash	Medical Officer	LPD, NIAID
Others:	M.M. Mowatt	Staff Fellow	LPD, NIAID
	D.H. Lujan	Visiting Fellow	LPD, NIAID
	L. Byrd	IRTA	LPD, NIAID
	P. Pimenta		LPD, NIAID
	A. Sher		LPD, NIAID

COOPERATING UNITS *(if any)*

B. Gottstein, Institute of Parasitology, University of Zurich, Switzerland; S. Erlandsen, University of Minnesota

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

An infection model of *Giardia lamblia* into adult mice has been developed. Only one isolate of nine studied consistently infected adult mice. Seven mice strains were readily infected but showed different courses of infections. The course of infection, changes in expressed VSP over time, and humoral responses were noted in a number of strains of mice as well as SCID and nude mice. Large numbers of trophozoites were present in the small intestines 1 - 2 weeks after inoculation. This was followed by a dramatic reduction in the numbers of trophozoites in the intestines. Two of three strains remained chronically infected, while a third was cured. SCID mice failed to show a reduction in the number of intestinal trophozoites over time. This model system allows the analysis of host-parasite interactions, especially those responses important in antigenic variation, maintenance of chronic infections, and cure.

The metabolism of cysteine in *Giardia* was studied. *Giardia* could neither manufacture cysteine *de novo* or convert other sulfur-containing compounds to cysteine. Two mechanisms appeared responsible for uptake, one of which was non-saturable. Oxidation of cysteine to cystine occurs readily in culture and can be prevented by the use of a Cu^{++} chelating compound, batocuproine.

IGF II, a component of Cohn fraction IV-1, is an essential growth-promoting factor of *Giardia*. Recombinant protein supported growth and cysteine uptake in the absence of serum, a necessary component for culture.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00162-17 LPD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.M. Dwyer	Supervisory Microbiologist	LPD,NIAID
Other:	M.A. Alleman	IRTA Fellow	LPD,NIAID
	A. Debrabant	Visiting Fellow, FIC	LPD,NIAID
	J.A. Dvorak	Supervisory Microbiologist	LPD,NIAID
(attached)	C. McCarthy-Burke	Biologist	LPD,NIAID

COOPERATING UNITS *(if any)*
CBER,DBB,FDA, (M. Joshi, G. Pogue & H. Nakhasi); DMID,NIAID (M. Gottlieb); Haskins Labs, Pace Univ. (C. Bacchi); Dept. Biochem., Univ. Kentucky (S.Turco); London School Tropical Med & Hyg (R. Harris & P. Kaye).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology & Cell Biology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	4.3	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cell and molecular biology of both *Leishmania* and *Toxoplasma* are investigated as models of intracellular parasitism. Emphasis is placed on characterizing both the biochemical functions and gene structure of their surface membrane (SM) and secreted proteins toward defining the roles of these constituents in parasite survival and development.

A gene encoding the unique, trypanosomatid surface membrane enzyme, 3'-nucleotidase has been identified, cloned and partially sequenced from *L. donovani*. Similarly, full length genomic clones of an *L. donovani* secretory acid phosphatase gene are being sequenced. The fate of this enzyme within infected macrophages is also being investigated. Methods were devised for the continuous *in vitro* cultivation of large quantities of infectious amastigotes (Am-) from *L. donovani* and several other species. These Am- are being characterized with regard to their cell biology and biochemistry. At the molecular level, several genes have been identified which are uniquely or differentially expressed by Am- and these are being used as probes to study parasite-gene-regulated differentiation and cell cycle development. Further, physiologic studies have demonstrated that both *Leishmania* and *Crithidia* use methylation as a means for the rapid and reversible modification of their surface membrane proteins *in situ*. Finally, a unique 5'-nucleotidase was identified in the surface membrane of *Toxoplasma gondii* tachyzoites and a PCR-generated probe is being used to identify the gene for this protein.

The current results have identified potential targets which are of relevance toward the development of new/improved diagnostic, chemotherapeutic and immunoprophylactic agents against these important human pathogens.

(Others cont.)

J.P. McDaniel	Biologist	LPD,NIAID
P.F.P. Pimenta	Guest Investigator	LPD,NIAID
G.J. Russell	Visiting Fellow, FIC	LPD,NIAID
D.L. Sacks	Supervisory Microbiologist	LPD,NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00197-14 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulation and Immune Recognition in Filariasis and Non-filarial Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.B. Nutman	Senior Investigator	LPD, NIAID
Others:	E.A. Ottesen	Senior Investigator	LPD, NIAID
	S. Mahanty	Senior Staff Fellow	LPD, NIAID
	L. Elson	Visiting Fellow, FIC	LPD, NIAID
	S. Mollis	HHMI Research Scholar	LPD, NIAID
	P. Zimmerman	NRC Fellow	LPD, NIAID
	S. Shaw	Senior Investigator	EIB, NCI

COOPERATING UNITS *(if any)*

Department of Allergy and Clinical Immunology, Univ. of Montreal, Montreal, Canada (G. Delespesse); Dept. of Clinical Investigation, Hospital Vozandes, Quito, Ecuador (Dr. R. Guderian); Univ. National de Benin (Dr. Massouboudgi), Anna Univ., Madras, India (Dr.K. Jayaraman)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.4	2.4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to delineate the mechanisms involved in regulating immune responses in filarial and nonfilarial disease states. Immunoregulatory studies have examined the phenomenon of antigen-specific anergy in microfilaremic patients by showing this anergy to be a result of a diminished frequency of proliferating cells to parasite antigen and of the production of the antiproliferative cytokines, IL-10 and TGF-β. Filter immunoplaque assays for the major cytokines have been developed and used to demonstrate that in helminth infections of humans there is an expansion of Th2 CD4+ cells. The phenotypic characterization of these Th2 CD4+ cells has shown them to be CD45RO+ HLA-DR+ CD27-. The underlying genetic basis for the immune responses to certain parasite antigens is currently being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00246-10 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Studies on the Genome and Surface of *Schistosoma mansoni*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alan Sher	Section Head	LPD, NIAID

COOPERATING UNITS *(if any)*

Molecular Vaccines, Inc., Gaithersburg, Maryland

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00251-12 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR	A. Sher	Section Head	LPD, NIAID
Others	I. Oswald	Special Volunteer	LPD, NIAID
	M. Williams	Special Volunteer	LPD, NIAID
	J. Actor	IRTA Fellow	LPD, NIAID
	T. Wynn	IRTA Fellow	LPD, NIAID
	A. W. Cheever	Assistant Chief	LPD, NIAID
	S. L. James	Program Officer	MIDP, IRP, NIAID

COOPERATING UNITS *(if any)* Others, from above: I. El Toum, Visiting Fellow, LPD, NIAID; R. Seder, Senior Staff Fellow, LPD, NIAID; Fiocruz, Recife, Brazil (A. Coutinho, S. Montenegro).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

In work completed this year, endothelial cells were shown to be activated by cytokines to kill schistosomula in culture. This effector mechanism was shown to require the production of nitrogen oxides and to be inhibitable by IL-4. An *in vivo* role for endothelial effector cells was suggested by the altered morphology of pulmonary endothelium in vaccinated mice rejecting challenge infections and the elevated expression of NO synthase mRNA.

Studies on the regulation of egg pathology in mice indicated that IFN-γ downregulates both egg-specific Th2 cytokine responses and granuloma formation. NK cells were implicated as the endogenous source of the IFN-γ. IL-12, through its induction of IFN-γ, was shown to be a potent down-regulator of granuloma formation in both liver and lung.

In a field study performed in Recife, Brazil, PBMC from patients infected with *S. mansoni* were shown to produce a predominant Th2 cytokine response to parasite antigens and mitogens, with minimal Th1 responses displayed to egg antigens. In this respect, the human cytokine response was found to closely resemble that previously described by us in mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00253-12 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Studies of Immunological Responses to Filarial Infections

PRINCIPAL INVESTIGATOR	E. A. Ottesen	Section Head	LPD, NIAID
Others	T. B. Nutman	Senior Investigator	LPD, NIAID
	N. Raghavan	Visiting Associate	LPD, NIAID
	S. Mahanty	NRSA Fellow	LPD, NIAID
	J. McCarthy	Visiting Fellow	LPD, NIAID
	L. Elson	Visiting Fellow	LPD, NIAID
	S. Mawhorter	Medical Staff Fellow	LPD, NIAID
	S. Williams	IPA Fellow	LPD, NIAID

COOPERATING UNITS *(if any)* Indian Council of Medical Research, Madras, India (S. Tripathy, V. Kumaraswami); Anna University, Madras, India (K. Jayaraman); CPqAM/FIOCRUZ, Recife, Brazil (G. Dreyer, A. Coutinho); University of Alabama (D. O. Freedman); (See below)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	2.1	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

(Cooperating Units Cont.) Quito, Ecuador (R. Guderian); Health Department, Cook Islands (A. Guinea); James Cook University, Queensland, Australia (P. Turner)

The goals of this project are to define the immunologic mechanisms responsible for resistance to filarial infection, for the acquisition of filarial disease, and for the pathological reactions that develop in patients being treated for these infections.

Differential immune responsiveness to filarial antigen among groups of individuals from areas endemic for bancroftian filariasis (Cook Islands, India) has shown generalized enhanced immunologic responsiveness in 'putatively immune' individuals. Screening of antibody responses identified at 43 kD larval antigen differentially recognized by the immune group; this antigen has been subsequently isolated from a *W. bancrofti* DNA expression library, the gene cloned, and the molecule identified as filarial chitinase (now a vaccine candidate).

A 17 year follow-up study in the Cook Islands provided the opportunity to identify long-term effects of prenatal exposure to maternal filarial infection on subsequent immune responsiveness to filarial antigens. Studies of lymphocyte and antibody responses of children born 17 - 19 years previously to mothers diagnosed at that time as either microfilaremic or amicrofilaremic showed that children of infection-free mothers (*i.e.*, those with no prenatal exposure to filarial antigen) had significantly greater responses to microfilarial antigen than did children born to microfilaremic mothers. Prenatal 'tolerization' is the most likely explanation for this finding. Following treatment of filarial infections, there is often an acute inflammatory reaction and a post-treatment eosinophilia. The eosinophilia has been shown to depend on the cytokine IL-5 probably released from peripheral blood T cells. This severe clinical reaction (Mazzotti reaction) has now been linked to inflammatory pathways characterized by the presence of IL-6 and TNF-α in the blood of affected patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00255-11 LPD

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Immunologic Responses to Non-Filarial Parasitic Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Section Head	LPD, NIAID
	T.B. Nutman	Senior Investigator	LPD, NIAID
	S. Mahanty	NRSA Fellow	LPD, NIAID
	N. Raghavan	Visiting Scientist	LPD, NIAID
	F.A. Neva	Laboratory Chief	LPD, NIAID

COOPERATING UNITS *(if any)*

CPqAM/FIOCRUZ, Recife, Brazil (G. Dreyer, A. Rochas)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasitic Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Terminated 1992 - 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00256-12 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Develpmental Biology of *Leishmania* Promastigotes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Sacks	Senior Investigator	LPD/NIAID
Other:	P. Pimenta	Guest Researcher	LPD/NIAID
	R. Kenney	Senior Research Investigator	
	E. Saraiva	Guest Researcher	

COOPERATING UNITS *(if any)*

Dr. Sam Turco, Dept. of Biochemistry, University of Kentucky
Dr. Stephen Beverley, Harvard Medical School, Boston
Dr. Ed Rowton, Walter Reed Army Institute of Research, Washington

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

During growth within the sandfly and within axenic culture, *Leishmania* promastigotes undergo differentiation from a dividing noninfective stage to a resting infective or metacyclic stage which is uniquely adapted for life in the vertebrate. This development is accompanied by a substantial modification of the surface lipophosphoglycan (LPG) which is the major surface glycoconjugate of these cells. In the case of *L. major* and *L. donovani* promastigotes derived from culture, the structural polymorphism is expressed as a loss of terminal galactose residues on the LPG. Using stage specific monoclonal antibodies against *L. major* LPG, identical structural changes in LPG have now been demonstrated for metacyclics found within the natural vector, *P. papatasi,* and in particular those metacyclics found to emerge from the proboscis during force feeding experiments. The substitution or capping of terminally exposed sugars with other sugar residues was shown to be the molecular mechanism underlying the attachment and release of promastigotes from midgut epithelial cells during metacyclogenesis in the fly. The interspecific polymorphisms of these terminal sugars appears to influence the species specificity of vectorial competence observed in nature. Thus, the ability of procyclic promastigotes or the purified procyclic LPG of various *Leishmania* species to bind to midgut epithelial cells of various sandfly species forcibly predicts vectorial competence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00257-12 LPD

PERIOD COVERED

October 1, 1992 to Sept. 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. A. Neva, Chief, LPD, NIAID
Others:	C. Brown, LIR, NIAID
	A. Gam, LPD, NIAID
	N. Raghavan, Visiting Associate, LPD, NIAID
	T. Lehky, NI, NINDS
(See below)	T. Nutman, Senior Investigator, LPD, NIAID

COOPERATING UNITS *(if any)*

Bioqual, Inc., Rockville (T. Moskal); Projet SIDA, Kinshasa, Zaire (J. Perriens); Northwestern Univ., Evanston, Illinois (P. Gann); Univ. of the West Indies, Jamaica (R. Robinson, Prof. O. Morgan, and S. Terry); Center Trop. Diseases, Univ. Mass at Lowell (J. Bruce)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	0.9	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

(Others Cont.)

E. Ottesen, Senior Investigator, LPD, NIAID
T. Nash, Senior Investigator, LPD, NIAID

This project deals with both the laboratory and clinical aspects of infection caused by the intestinal nematode, *Strongyloides stercoralis*. The laboratory research involves analysis and characterization of parasite antigens. The clinical studies focus upon the immune response of infected individuals, and factors that influence the immune response.

A cDNA library was made in a λgt II phage expression vector from mRNA extracted from third stage larvae of *S. stercoralis*. Two clones were identified which reacted with antibodies from a pool of patients' serum infected wiht the parasite. Recombinant material expressed by the clones in *E. coli* reacted by Western blot to both IgG and IgE from most individual sera of infected patients. Attempts are under way to express the clones in another vector to obtain better sequence data, and more clones will be sought from the cDNA library.

In one of the main clinical studies during the past year, the efficacy of Ivermectin was compared to that of the standard treatment with thiabendazole in a group of 53 Cambodian refugees now living in Lowell, Massachusetts. Only one treatment failure occurred in 18 thiabendazole-treated patients, and one failure in 35 Ivermctin-treated cases. Ivermectin was very well tolerated, but side effects were frequent with thiabendazole. The association of persistent strongyloides infection with HTLV-1 is still under investigation.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00347-11 LPD

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.W. Cheever	Head, Host-Parasite Relations, LPD, NIAID
Others:	D. Abrams	Bio. Lab. Tech., LPD, NIAID
	R. Poindexter	Bio. Lab. Tech., LPD, NIAID
	I. Eltoum	Visiting Fellow, LPD, NIAID
	A. Sher	Head, Immunology Sect., LPD, NIAID
	T. Wynn	Immunology Sect., LPD, NIAID

COOPERATING UNITS (if any)

Department of Medicine, USUHS (Fred Finkelman) and USDA (Joseph Urban). Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brazil (Zilton Andrade).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, Md 20892

TOTAL STAFF YEARS: 2.1	PROFESSIONAL: 1.5	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Morbidity in schistosome-infected individuals is caused mainly by the immune response of the host to schistosome eggs deposited in the tissues. In chronic schistosomiasis, fibrotic sequelae to the inflammatory reaction to the eggs are responsible for most clinical disease.

Hepatic fibrosis and the granulomatous response to eggs of schistosome species pathogenic for man are studied in mice in relation to parasitologic parameters of infection. Cytokines play an important role in the genesis and regulation of the size of circumoval granulomas and in the fibrosis associated with them. Treatment of mice with antibodies to IL-4 prior to egg laying markedly decreased hepatic fibrosis in *S. mansoni* and *S. japonicum*-infected mice while having trivial effects on the size of granulomas around the schistosome eggs. However, anti-IL-4 treatment of naive mice injected intravenously with schistosome eggs inhibited the development of pulmonary granulomas.

Our results suggest that Th2-type cell responses are important for the fibrotic responses to schistosome eggs and, by analogy, perhaps for other hypersensitivity reactions in which "delayed hypersensitivity" is important.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00350-11 LPD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT
DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR

P.I.	T. E. Nash	Medical Officer	LPD, NIAID
Others:	M. M. Mowatt	Staff Fellow	LPD, NIAID
	R. Adam		University of Arizona

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

A PCR technique employing relatively conserved regions of variant-specific surface antigen (VSP) genes was developed to differentiate *Giardia* isolates. The regions of the VSPH7 protein responsible for Zn and metal binding was determined by the use of fusion peptides containing various regions of VSPH7. Although the previously defined LIM-RING finger-like region bound Zn, other regions also could bind metals, and this was most likely due to CXXCXnCXXXC motifs scattered throughout the protein. There is conservation of spacing between CXXC motifs in a subset of VSPs. These findings indicate a conservation of structure, but diversity in antigenicity. Prior studies identified a transcript (GLORF-C4) specific for Group 3 *Giardia*; however, antibodies produced to the fusion protein identified a similar protein in other groups. Therefore, homologous proteins are present in other *Giardia* groups, and the reason the transcripts are not identified is because the sequences differ. Other proteins have also been identified which differ among *Giardia lamblia* isolates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00439-09 LPD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Clinical and Therapeutic Studies of Human Filariasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Section Head	LPD, NIAID
Others:	S. Mahanty	NRSA Fellow	LPD, NIAID
	S.A. Williams	IPA Fellow	LPD, NIAID
	J. McCarthy	Visiting Associate	LPD, NIAID
	P. Zimmerman	NRC Fellow	LPD, NIAID
	L. Elson	Visiting Fellow	LPD, NIAID
	N. Raghavan	Visiting Associate	LPD, NIAID

COOPERATING UNITS *(if any)*
Indian Council of Medical Research, Madras, India (S.P. Tripathy, V. Kumaraswami, R. Prabhakar); MGR Medical College, Madras, India (V. Vijayasekaran); Peace Corps Medical Office (C. Waterman); Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil (A. Countinho, G. Dreyer).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Clinical Parasitology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER.
1.3	0.9	0.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical studies in lymphatic filariasis have defined the persistence of microfilaremia in patients for decades without leading to overt clinical pathology, have identified individuals apparently immune to infection, and have detected subclinical renal and lymphatic pathology in 'asymptomatic' microfilaremic patients. In both loiasis and onchocerciasis, studies on expatriate and endemic populations have defined the hyperresponsive clinical features of the expatriates that contrast with those of endemic populations.

Increased diagnostic sensitivity and specificity have been achieved by using PCR-based amplification of parasite DNA in skin snips from patients with onchocerciasis and blood specimens from patients with bancroftian filariasis.

Therapeutic trials of ivermectin in bancroftian filariasis have indicated its superiority to diethylcarbamazine as a microfilaricide, and further studies are underway to compare its macrofilaricidal and synergistic activities with DEC. Trials of albendazole in loiasis indicate that this drug appears to be an effective macrofilaricide that can be given to patients more safely than DEC.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00487-07 LPD

PERIOD COVERED October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Quantitative Parasitology of Schistosome Infections.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A.W. Cheever, Chief, Host-Parasite Relations Section

Others:	D. Abrams	Bio. Lab. Tech., LPD, NIAID
	R. Poindexter	Bio. Lab. Tech., LPD, NIAID
	J. Mosimann	Biostatistician, ORI
	Z. Andrade	Fogarty Scholar
	J. Actor	Immunology section, LPD, NIAID
	F. Lewis	Biomedical Research Institute

COOPERATING UNITS *(if any)*

Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brazil (Zilton Andrade). Biomedical Research Institute, Rockville, MD (Fred Lewis).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Morbidity in schistosomiasis is caused by the host response to schistosome eggs deposited in the venous system and carried to the tissues. Pathology is proportional to the number of eggs laid. It is thus important to understand factors underlying worm fecundity and the extent to which fecundity is reflected by eggs passed in the feces, the measurable indicator of infection intensity in humans.

Treatment of mice with anti-TNF-α resulted in slightly smaller circumoval granulomas and slightly decreased egg laying in *S. mansoni* but not *S. japonicum* infections. Antibodies to TGFß, IL-10 or IL-6 in *S. mansoni* infected mice did not affect worm fecundity. Egg laying was also normal in *S. mansoni*-infected $CD8^+$ and IL-10 knockout mice.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00494-07 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Analysis of T Cell Responses in Human Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sacks	Senior Investigator	LPD,NIAID
Others:	R. Kenney	Senior Research Investigator	LPD,NIAID
	L. Carrera	Guest Research	
	F. Neva	Chief	LPD,NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parisitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS	PROFESSIONAL	OTHER.
2.8	2.8	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The critical immunological feature of visceral leishmaniasis is the complete absence of cell mediated immunity to leishmanial antigens. Patients have been shown to have negative intradermal skin tests to Leishmanin, absent lymphocyte blastogenesis, and decreased IL-2 and IFN-γ production in response to parasite antigens. Experimental models of visceral leishmaniasis suggest that the disease is characterized not by the lack of cytokine production per se, but by the production of potentially counter-protective cytokines. We sought to maximize assay sensitivity by using semi-quantitative RT-PCR techniques to analyze cytokine mRNA extracted from lesional tissue (bone marrow). In preliminary data we provide evidence that IL-10 mRNA is present at relatively high levels in active kala-azar, being at or below the limits of detectability after treatment, and absent in uninfected controls. The cellular source of this potentially down-regulatory cytokine is being investigated in patients from India by repeating the above experiments using bone marrow cells positively and negatively selected for B cells, T cells, or monocytes using magnetic beads.

In *in vitro* MØ infections, procyclics are killed by MØ several hours post infection whereas metacyclics survive, transform to amastigotes and replicate. These different outcomes provide a good model for studying cellular responses elicited during infection that are associated with either intracellular killing or survival. *L. major* promastigotes induced remarkably weak cytokine responses by mouse macrophages during the early stage of infection and no dramatic differences were observed in the induction of cytokine mRNA expression by procyclic and metacyclic promastigotes. Both procyclic and metacyclic promastigotes downregulated cytokine gene expression by LPS-treated macrophages. This downregulation was more efficient in the case of metacyclics and this could be related to their survival. Significant differences were observed between procyclics and metacyclics in the activation of the oxidative burst. It seems likely that the oxidative burst is the major mechanism involved in the killing of procyclic promastigotes. These results suggest that activation pathways leading to oxygen-dependent and independent killing mechanisms can be triggered independently in *Leishmania* infections.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00512-06 LPD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Molecular Definition of Filarial Antigens

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	T. B. Nutman	Senior Investigator	LPD, NIAID
Others:	N. Raghavan	Visiting Associate	LPD, NIAID
	James McCarthy	Visiting Associate	LPD, NIAID
	Lynne Elson	Visting Fellow, FIC	LPD, NIAID
(below)	Stacey Mollis	HHMI Research Scholar	LPD, NIAID

COOPERATING UNITS (*if any*)
New England Biolabs, Beverly, Mass (F. Perler, L. McReynolds, C. Maina), Department of Geographic Medicine, University of Alabama, Birmingham (D. Freedman), Swiss Tropical Institute (E. Lobos)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Clinical Parasitology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	3.6	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Steven Williams	IPA	LPD, NIAID
Peter Zimmerman	NRC Fellow	LPD, NIAID
E. A. Ottesen	Senior Investigator	LPD, NIAID

The objectives of this project are to define and generate filarial proteins that are important in inducing parasite-specific immune responses in the human host and to understand, at a molecular level, the differences among related filarial species. Recombinant antigens and probes have been identified that: a) encode immunoreactive and potentially protective molecules of *W. bancrofti*; b) can distinguish among related filarial species; c) identify repeated segments of *W. bancrofti* genome, *O. volvulus* or that of *Loa loa*; d) may be of potential diagnostic importance and e) induce immediate hypersensitivity type responses in lymphatic filarial syndromes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00579-04 LPD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Immune Regulation in Toxoplasmosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Senior Investigator	LPD, NIAID
Others:	R. Gazzinelli	Senior Staff Fellow	LPD, NIAID
	E. Denkers	IRTA Fellow	LPD, NIAID
	L. Aslund	Special Volunteer	LPD, NIAID

COOPERATING UNITS *(if any)*

Genetics Institute, Cambridge, MA (S. Wolfe); Wistar Institute Philadelphia, PA (G. Trinchieri, M. Wysocka)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall aim of this project is to analyze the immune response to *Toxoplasma gondii* in order to define which cellular immune components and parasite target antigens are involved in the control of infection and its breakdown in immunocompromised hosts.

Progress was made this year in the following areas:

A. *In vivo* analysis of macrophage functional markers and cytokine expression during reactivation of infection in brain tissue. Reactivation of *T. gondii* infection in mouse brain was shown to correlate with the disappearance of mRNA's encoding markers of macrophage activation and to be inducible by depletion of TNF-α.

B. Function of NK cells as alternative effectors of IFN-γ—dependent resistance. Studies on vaccine-induced resistance in CD8+ deficient mice indicated that NK cells can mediate immunity against *T. gondii* via the T independent production of IFN-γ.

C. Role of IL-12 in the induction by *T. gondii* of IFN-γ from NK cells and the use of the cytokine in the stimulation of resistance in immunodeficient hosts. IL-12 was shown to play a crucial role in the induction of IFN-γ synthesis in NK cells by *T. gondii*. Macrophage accessory cells were shown to be the source of the IL-12 induced by the parasite. When administered to immunodeficient mice, IL-12 was shown to induce partial resistance against *T. gondii* challenge.

PHS 6040 (Rev. 5/92)

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1993 Annual Report

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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1992 to September 30, 1993

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD this past year:

Arrivals:

Dr. Richard Bessen, from the University of Wisconsin, Madison, Department of Animal Health and Biomedical Science, working as an IRTA Fellow with Dr. John Portis and Dr. William Lynch.

Dr. Gil Katzenstein, from the University of North Carolina, Chapel Hill, North Carolina, working as an IRTA Fellow with Dr. Byron Caughey.

Dr. Marc Lavignon, from the Laboratory of Professor C. Paoletti, Villejuif, France, working as a Visiting Fellow with Dr. Leonard Evans.

Dr. Nancy Ray, from Oregon State University, Corvallis, Oregon, working as an IRTA Fellow with Dr. Donald Lodmell.

Dr. Kathy Toohey, from the University of Minnesota, working as an IRTA Fellow with Dr. Bruce Chesebro.

Departures:

Dr. Hiroyuki Kanno, Visiting Fellow, returned to the Department of Pathology, Osaka University, Medical School, 2-2 Yamadaoka, Suita, OSAKA 565, JAPAN

Summer students were John Courchesne from University of Montana, Missoula, Montana; Brett Denhart from Harvard University, Boston, Massachusetts; Mark Huhtanen from Montana State University, Bozeman, Montana; David Martin from the University of Rochester Medical School, Rochester, New York; James Nolz from University of Nevada, Reno, Reno, Nevada; Shelly Robertson of Washington State University, Pullman, Washington; and Shannon Waliser of Caroll College, Helena, Montana.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1992 to September 30, 1993

OVERVIEW OF RESEARCH IN LPVD

Research at LPVD is aimed at understanding mechanisms of virus persistence and latency with particular emphasis on immune surveillance by the host and virus evasion of host defenses operating in different systems.

In the case of murine retroviruses, the following areas are being studied:

Characterization of host genes and immune mechanisms involved in recovery from Friend virus-induced erythroleukemia, use of vaccinia-retrovirus recombinants to induce protective immunity, use of retrovirus expression vector systems to study cell tropism of infection and to characterize viral epitopes recognized by the host immune response. (Chesebro, Hasenkrug)

Study of degenerative central nervous system disease induced by mouse retroviruses including characterization of viral gene sequences influencing cell tropism, disease kinetics and pathogenic events at the light and electron microscopic level. (Portis, Lynch, Bessen)

Study of the specificity of generation of recombinant retroviruses by interactions between exogenous viruses and endogenous viral sequences. Characterization of the role of recombinant retroviruses in leukemogenesis and autoimmunity. (Evans, Lavignon)

Studies are also in progress on human and equine lentiviruses (HIV and EIAV). We have developed a focal *in vitro* infectivity assay for these viruses. Cell surface receptors and other cellular factors influencing cell tropism of infection are being studied. AIDS patient HIV isolates with unusual cell tropism are being examined by PCR amplification and cloning to determine influence of specific viral gene sequences on cell tropism. Pseudotypes of HIV genomes with envelope proteins of other human or murine retroviruses are being studied for patterns of altered tropism *in vitro* and *in vivo*. The possible role of HIV strain differences in induction of the HIV dementia syndrome is being investigated by cloning and sequence of viral genes obtained from CNS tissue of defined clinical populations. In the EIAV system viral and cellular specificity of the transactivation process is being studied and compared to results using HIV and HIV/EIAV chimeric promotors and transactivating (tat) genes. (Chesebro, Maury, Toohey)

Other virus systems being studied include rabies virus in mice, Aleutian disease virus of mink, and the hamster and mouse-adapted strains of the scrapie agent.

In the murine rabies model, host genes have been found to influence ability of mice to recover from infection. In some mouse strains, recovery can occur even after CNS infection and clinical symptoms have developed. The immune mechanisms of this recovery are the main focus of study. Mechanisms of immune protection are also being investigated after vaccination with recombinant pox viruses expressing rabies virus envelope or nucleoproteins. (Lodmell, Ray)

Aleutian disease virus (ADV), a parvovirus, has been investigated in adult mink as an infectious model of the human disease systemic lupus erythematosus and in newborn mink as an infectious model for the hyaline membrane-associated respiratory disease of newborn humans. In the mink there are susceptibility and resistance genes in the host as well as virus strains which vary in virulence and host tropism. Current efforts at LPVD have resulted in molecular cloning of several virus strains and DNA sequencing is underway to determine which genes control virulence. More recently, strand-specific

ADV probes have been used in *in situ* hybridization to distinguish cells replicating ADV from cells where ADV virions accumulate during disease. In addition, monoclonal antibodies specific for individual virus strains have been developed and are being used to characterize sites of virus replication and virus-antibody complex deposition *in vivo.* (Kanno, Bloom)

Epidemiology and pathology of the scrapie agent were studied by the slow virus group at RML for many years. Previously we cloned and sequenced a cDNA copy of the mRNA encoding the mouse scrapie-associated PrP protein. This gene was found to be expressed in both infected and uninfected animals, suggesting that this protein is not virus-encoded, but instead may be a normal brain protein involved in amyloid deposition during scrapie pathogenesis. Recently emphasis has switched to development of *in vitro* culture systems for scrapie. Neuroblastoma cell lines persistently carrying the transmissible agent have been developed, and cell cloning has yielded cultures where almost all cells are "infected". Current efforts are directed towards biochemical characterization of the generation of the agent-associated protease-resistant amyloid-like PrP protein in these cells. (Race, Chesebro, Caughey, Priola, and Doh-Ura)

Amyloid has also been the focus of studies in a nonviral model in hamsters. A hamster serum protein under sex hormonal control was identified and found to be a component of spontaneous and induced amyloid in hamsters. During experimental hormonal manipulation of hamster serum proteins, estrogens were found to induce hepatomas in hamsters. Possible role of oncogene- or growth factor receptor-mediated mechanisms of tumorigenesis are being studied. (Coe, Satoh)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00072-22 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. L. Lodmell	Scientist Director	LPVD, NIAID
Other:			
	Nancy B. Ray, Ph.D.	IRTA Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
Joseph J. Esposito, Ph.D. CDCP

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The principal objectives of this research project are five-fold: 1) to determine host and viral factors which influence the genetically controlled resistance of inbred mouse strains to street rabies virus (SRV) 2) to determine if an *in vivo* laboratory model for persistent rabies virus infection can be established with subsequent goals of determining in which cell(s) the virus (genome) is sequestered and whether the virus can be activated to produce clinical disease and death 3) to understand the role of macrophages in rabies virus infections 4) to compare the protective capabilities of recombinant vaccines expressing different or multiple structural proteins of the rabies virus against genetic variants of rabies virus that have been isolated worldwide and 5) to determine if sera from mice vaccinated with a recombinant virus expressing the internal structural rabies virus nucleoprotein is protective and if so, the mechanism of the protection.

Studies with recombinant vaccines expressing either the rabies virus glycoprotein (G) or nucleoprotein (N) are continuing. *In vitro* and *in vivo* models to determine if the serum of mice vaccinated with recombinants expressing the internal structural N protein is protective have been established. *In vivo* studies illustrated that mice inoculated in the footpad with preincubated mixtures of anti-N sera and virus were protected. In addition, anti-N sera inoculated into the site of virus challenge protected mice. The neutralizing activity of anti-N sera also was demonstrated *in vitro*. Infectious virus was not detected in cultures 24 hours following infection with virus that had been preincubated with anti-N sera. At later time points infectious virus was detected, but inhibition of viral replication was consistently $\geq$ 99% compared to control cultures. The protective and neutralizing activity of the anti-N sera was identified as antibody by several methods. First, absorption of anti-N sera with goat anti-mouse immunoglobulin serum, but not normal goat serum, removed the protective activity. Second, radioimmunoprecipitation and SDS-PAGE separation of sucrose density gradient fractionated anti-N sera showed that anti-viral activity was present only in the fraction containing anti-N antibody. Finally, absorption of anti-N sera with insect cells infected with a baculovirus expressing the N protein removed the protective activity. These data indicate that anti-N antibody is an important component of resistance to rabies virus infections. Because of the highly conserved sequence of the N protein of different rabies virus isolated worldwide we suggest inclusion of N with G in future recombinant rabies vaccines, and inclusion of antibodies to both proteins in antibody mixtures used in rabies immunoglobulin serotherapy.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00074-21 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetically Controlled Mechanisms of Pathogenesis and Recovery in Friend Retrovirus-Induced Leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	K. Hasenkrug	IRTA	LPVD, NIAID

COOPERATING UNITS *(if any)*
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews
This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Friend virus (FV) is a retroviral complex composed of a replication defective virus, SFFV, and a replication competent helper virus, F-MuLV, that causes erythroleukemia in susceptible strains of adult mice. Because FV infects immune competent adult mice, the host immune response has the potential to influence the disease outcome. Previously we have identified mouse strains fully susceptible to FV infection which were still capable of spontaneous recovery from both infection and established erythroleukemia. Other strains could recover from infection, but still succumbed to leukemia. Yet other strains varied in their ability to mount a protective immune response following vaccination. In all these situations we have attempted to identify host genes which influence the disease and to characterize their mechanisms of action. So far, we have found four genes in the major histocompatibility complex (MHC) in the D, IA, IE and T regions, and one non-MHC gene, Rfv-3, which influence various aspects of the host immune response to FV. One strain in particular shares some interesting host-response characteristics with HIV infections. These mice mount virus-specific humoral responses in the face of severe virus-induced immunosuppression such as that seen in AIDS patients. As in AIDS patients, these virus-neutralizing antibody responses are inadequate for protection. However, antibody does appear to cause clearance of viremia and a marked decrease of infectious virus in the cells of infected tissues such as spleen.

The main goals of this project are to determine the types of immune responses required for recovery from Friend disease, to determine the fine specificities of those responses, to determine how protective immunity and/or spontaneous immune responses might be modified to improve recovery, and finally, to determine the mechanisms by which genetic factors influence those responses.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00085-16 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Aleutian Disease Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Other:	H. Kanno	Visiting Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.3	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical IIDEA project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The scope of this project is to elucidate pathogenic mechanisms involved in infections of mink with Aleutian mink disease parvovirus (ADV).

The infection by ADV of the monocyte cell line U937 was shown to be dependent upon antiviral antibody, a phenomenon known as antibody dependent enhancement (ADE). In the presence of antibody both the pathogenic ADV-Utah 1 and the nonpathogenic ADV-G could infect U937 cells. This effect required an intact Fc portion of the IgG molecule. This infection was restricted in that although viral DNA, RNA and protein synthesis occurred, infectious virus was not generated. Specific differences in mRNA species were noted between permissive infection of CRFK cells and infection of the U937 cells by ADE. Similar results were noted in primary mink macrophages, but studies were limited due to availability of these cells.

ADE infection of U937 cells by ADV stimulated the production of human interleukin-6 (IL-6) and IL-6 mRNA, indicating that a restricted ADE-mediated infection of these cells could induce this potent immune modulator. A portion of the mink IL-6 gene was cloned by PCR from mRNA from mink cells. The sequence was ≈70% homologous to human IL-6 at the DNA level. Conditions in which IL-6 is overproduced in human and mice resemble the clinical and pathologic picture of ADV infection in adult mink, suggesting that IL-6 may have a role in Aleutian disease.

PHS 6040 (Rev 1/84)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00086-16 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Portis	Medical Officer	LPVD, NIAID
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	Richard Bessen	IRTA Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	3.0	1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The retroviruses we study were isolated from wild mice and cause a non-inflammatory neurodegenerative disease in mice similar to that caused by the unconventional agents such as scrapie. We are using this animal model to study the pathogenesis of retrovirus-induced neurodegeneration. Viral envelope protein: The neurovirulence of these viruses is dependent on the viral envelope gene, but the nature of this effect is not known. Virus replication occurs in both neuronal and non-neuronal tissues, although lesions are noted only in neuronal tissues. Interestingly immunoblot analysis has revealed a striking difference in the viral envelope protein expressed in neuronal and non-neuronal tissues of mice neonatally inoculated with FrCas^E. The envelope protein expressed in the brain exhibits two forms a 70kd and a ca. 64kd species, the later of which predominates coincident with the appearance of lesions and is aberrantly glycosylated. *In vitro* studies with purified populations of cells from the brain reveal that the two different species of envelope protein are likely produced by different cell types in the brain. The relevance of this aberrant viral protein to disease pathogenesis has yet to be understood. *In vitro* model of neurodegeneration: The neuronal cytopathology induced by FrCas^E and related retroviruses appears to be caused by an indirect mechanism probably requiring cellular interactions in the brain. Although an *in vitro* model by which to study at the molecular level the mechanism of neurodegeneration is highly desirable, we and others have been unable to demonstrate neuronal cytopathology as a consequence of virus infection of neuronal cell lines *in vitro*. In contrast to culturing individual cell types, organotypic slice cultures of the brain allow the maintenance of the architectural integrity of the brain as well as the full complement of cell types for extended periods of time. We have been successful at culturing brain slices from mice infected with FrCas^E, have demonstrated that these cultures are persistently and productively infected and are beginning to define the cell types which express virus and whether lesions evolve *in vitro*. Mechanism of neurodegeneration: A variety of cell types in the brain are infected by FrCas^E. We are currently culturing purified populations of astrocytes, microglial cells and endothelial cells which are infectable *in vitro*. Preliminary studies indicate that some of these infected cells when transplanted into the mouse brain, take up residence and persist. This system is now being utilized to study the cellular interactions which are involved in the induction of neurodegeneration.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00262-12 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Pentraxins in Hamster Physiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. E. Coe Medical Officer LPVD, NIAID

Other:

COOPERATING UNITS *(If any)*
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	.75	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Serum of Syrian hamsters contains a remarkable protein called female protein (FP) that is preferentially expressed in the female hamster. Accordingly, female Syrian hamsters normally have 2-3 mg FP/ml serum whereas normal males have levels 200-300 fold less. This protein is synthesized in liver, and has an unusual structure for a serum protein in that it is an oligomer of 5 identical monomeric subunits arranged as a cyclic pentamer. This protein is homologous to two human proteins, C-Reactive protein (CRP) and amyloid P component (AP), and shares 50-70% (respectively) identical amino acid sequence. This family of proteins is called pentraxin. FP shares similar functional properties with CRP and AP such as Ca++ dependant Phosphorocholine and galactan binding, complement fixation and acute phase responsiveness. Pentraxins are widely expressed in nature, and are found even in very primitive animals as horse shoe crab. The conservative evolution of this protein family and its ubiquitous expression suggests that pentraxins are biologically important. However, a critical role for these proteins has not been found. The unusual endocrine control of FP in hamsters suggests that this pentraxin may have some unusual function in the hamster which could provide information about the general function of all pentraxins. So far we have not found any beneficial effect from FP, and in fact high synthesis rates of FP appears to result in a shortened longevity of the female hamster. In Syrian hamsters, females normally die at an earlier age than males, (in contrast to all other mammals) and female hamsters generally die because of amyloidosis. FP of Syrian hamsters is a constituent of amyloid (similar to AP in human amyloid), and we have shown that high serum levels of FP appear to have a primary role in the induction of amyloidosis in Syrian hamsters. The endocrine control of FP synthesis is different in other hamsters. Thus, serum FP concentration is lower in Turkish hamsters and this species, which is very closely related to Syrian hamster does not acquire amyloidosis. Turkish hamsters appear to have an FP with identical amino acid sequence to Syrian hamster FP, although serum FP levels in females are about 10% that found in Syrian hamsters. Control of FP synthesis in Armenian hamsters is especially different from Syrian hamster because estrogen actually down regulates Armenian FP synthesis. Exogenous estrogen also is extraordinarily toxic to livers of Armenian hamsters and chronic estrogen administration initiates in the appearance of hepatic neoplasms. Estrogen-induced hepatotoxicity (cholestatic jaundice of pregnancy) and hepatic cancer are known to occur in humans, but there are no comparable animal models to study these phenomena.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00263-12 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID

Other:

COOPERATING UNITS *(if any)*
Department of Veterinary Virology, Royal Danish Veterinary University, Copenhagen, Denmark

Department of Veterinary Microbiology, Iowa State University

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical IIDEA project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The ongoing purpose of this project is to relate structural features of the genome of ADV to the ability of various isolates to replicate in vitro or in vivo and to cause disease in infected mink.

We have prepared and studied additional clones that are chimeric for genomic segments between ADV-G (type 1 ADV) and ADV-Utah 1 (type 2 ADV). A 0.5 kb segment (54 and 63 map units) of a type 2 ADV can render a replication competent construction replication defective. This piece, which has 4 coding changes, is contained within the shared coding sequences of the capsid protein gene and a single G→D transition in this region has the same effect as exchanging the entire fragment. The block to replication is manifest by a defect in progeny strand production and operates through a cis-acting function which cannot be complemented in trans. Constructions in which the capsid gene was hybrid between type 1 and type 2 sequences exhibited decreased levels of capsid protein levels in infected cells, suggesting that capsid stability may play a partial role in explaining this phenomenon. An ADV-G clone in which expression of the small NS2 protein was ablated by directed mutagenesis was replication defective indicating that this gene product is required for replication in CRFK cells.

Prokaryotic expression vectors expressing portions of specific capsid and nonstructural open reading frames (ORFs) have been made. A product specific for NS2 was unreactive with antibody from infected mink.

Comparative analysis of terminal sequence from ADV-Utah 1 with ADV-G revealed a number of dinucleotide changes between strains that might also be important in determining cellular permissivity.

A recombinant vaccinia virus expressing only the ADV VP2 gene made proteins that self-assembled into viral particles, indicating that VP1 was dispensable for particle formation. Mink vaccinated with recombinant vaccinia viruses expressing VP1/VP2 or only VP2 were not protected against subsequent challenge with ADV-Utah 1.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00265-12 LPVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. E. Race	Research Veterinarian	LPVD, NIAID
Other:	B. Caughey	Staff Fellow	LPVD, NIAID
	S. Priola	Staff Fellow	LPVD, NIAID
	B. Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS *(if any)*

Dr. A. Haase, Chief, Dept. Microbiology, University of Minnesota, Minneapolis, MN; Dr. Al Jenny, U.S.D.A., Ames, IA; Dr. Lyle Miller, Iowa State University; Dr. Randall Cutlip, U.S.D.A.; Dr. Michael Oldstone, Scripps Clinic, San Diego CA

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	3.2	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Scrapie is a spongiform encephalopathy of sheep and goats which can be transmitted experimentally to several other animal species. Similar diseases are recognized in cattle and humans. No etiologic agent has been identified. However, the proteinase K resistant form (PrP-res) of an endogenous protein designated prion protein (PrP) purifies with infectivity and is important to disease pathogenesis.

We developed a sensitive assay for PrP-res and utilized it to diagnose scrapie in sheep. Analysis based on PrP-res detection was much more accurate and less subjective than the currently used method of diagnosis based on the microscopic evaluation of brain. We also showed that PrP-res analysis of spleen or lymph node was nearly as accurate as analysis of brain. We are determining if PrP-res accumulates prior to clinical disease in lymph node. If so, an ante mortem test for infection may for the first time be possible. We are also using PrP-res analyses to test tissues from cattle in order to determine if spongiform encephalopathy currently exists in U.S. cattle and, thereby, whether an epidemic similar to BSE in Great Britain is possible in the U.S.A. PrP-res analysis should also be relevant for diagnosis of the human disease counterparts.

The influence of specific PrP gene sequences on interspecies transmission of spongiform encephalopathies is also being studied. To do so, we have expressed various mouse-hamster PrP constructs in scrapie-infected mouse neuroblastoma cells and are now analyzing them in mice and hamsters to determine if species tropism has been altered. Similar experiments are being done *in vivo* using transgenic mice containing the hamster PrP gene within their germ line.

Mouse neuroblastoma cells (MNB) are being used to study the normal function of the PrP protein as well as to identify factors which might account for the biochemical changes which lead to the conversion of the endogenous PrP protein to the PrP-res form.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00266-12 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. H. Evans	Chemist	LPVD, NIAID
Other:	R. J. Monk	Senior Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Genetic variation is a hallmark of retrovirus infection and occurs in avian, murine and human retroviruses such as HIV. The major focus of this project is the study of genetic variation in retroviruses and the effect of such variation on virus infection and disease. Studies currently underway in the laboratory include the determination of the point mutation rate of retroviruses; the polymorphism of recombinant host range retrovirus variants and *in vivo* pseudotyping and interference as a result of mixed retrovirus infections.

A. Reported estimates of mutation rates of retroviruses suggest that progeny retroviruses sustain several point mutations during a replication cycle. In contrast we have determined that most progeny murine retroviruses do not sustain any point mutations during a single cycle in a fibroblastic cell line. Studies are initiated to determine the MuLV mutation rate in macrophages and also to compare the point mutation rate of viable progeny viruses to that of proviruses in the absence of selection for viability.

B. Inoculation of many murine retroviruses in mice results in the generation of host range variants by recombination of the inoculated virus with endogenous retroviral genes. We have identified two major antigenic subgroups of these variants distinguished by their reactivity with two monoclonal antibodies (mAbs). Via chimeric virus construction and mutagenesis we have identified a region of the envelope gene which encodes the determinants to these mAbs and defined a single amino-acid residue which influences reactivity to one of the mAbs. Current studies are directed at closely defining the residue(s) which determine reactivity to the other mAb and determining the distribution of endogenous sequences in the mouse genome which correspond to the two major antigenic subgroups of recombinant viruses.

C. The generation of host range variants in retrovirus-infected mice results in a mixed retrovirus infection which may give rise to viral pseudotyping. We have found this to be the case in mice infected with the Moloney lymphocytic leukemia virus. Moreover, the pseudotyping appears to be a dynamic phenomenon which varies in different organs, changes during the course of disease and may influence the spread of infection of the target organ by host range variants. We are currently attempting to specifically inhibit spread of the host range variants during disease to determine the effect on tumor progression.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00418-10 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular biology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Maury	Staff Fellow	LPVD, NIAID
Other:	B. Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The horse lentivirus, equine infectious anemia virus (EIAV), is a retrovirus closely related to the human immunodeficiency virus. EIAV is useful as a model for the study of retroviral expression and latency.

A. The influence of the virus-encoded, trans-activating protein, Tat, on EIAV LTR activity continues to be investigated. We have shown that EIAV Tat is able to upregulate EIAV gene expression in a number of some, but not all cell lines. Additionally, not all cell lines which support EIAV transactivation support HIV transactivation. Regions within the EIAV LTR and Tat peptide which are important in transactivation have been identified. In cells highly permissive for EIAV expression, the EIAV R sequences which contain the Tat binding site, TAR, were the only EIAV specific sequences required; HIV enhancer/promoter sequences were able to substitute for analogous sequences within EIAV despite the virtual absence of sequence homology. In contrast, both EIAV and HIV enhancer sequences were required for maximal transactivation in cells restricted for EIAV expression. Further mapping of the enhancer sequences required for transactivation are ongoing. In a related project, identification and characterization of EIAV enhancer binding proteins is underway. The enhancer sequences and the proteins which bind to those sequences in non-permissive and permissive cells and cell lines is being explored. At the present time, these studies involve electrophoretic gel shifting assays as well as transient transfectioun assays for detection of viral gene expression.

B. Latency and viral reactivation in an field infected, EIAV-seropositive mare is being investigated. This mare has never been clinically ill with EIAV. No infectious virus has ever been isolated from her, however, by immunocompromising the mare EIAV sequences have been detected by PCR in her peripheral blood monocytes. Further characterization of the latent state of EIAV is being performed. Despite continued high titers of anti-EIAV antibodies in this mare's serum, it is possible that the inability to isolate infectious virus from this mare is due to the absence of infectious virus in this animal at this time (i.e., 3 years post-infection). To determine if infectious EIAV is present in the peripheral blood of this mare, blood from this mare has been introduced into a naive horse. This study should allow us to determine the infectivity potential.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00468-08 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Human AIDS Retrovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	W. Maury	Staff Fellow	LPVD, NIAID
	K. Toohey	IRTA Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.4	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Human immunodeficiency virus (HIV) is known to cause a variety of clinical syndromes including immunosuppression, dementia and a "wasting" syndrome. Extensive variation in the nucleic acid sequence of HIV isolates from various individuals has been observed, and this heterogeneity of viruses might account for the variations in disease course and symptomatology in infected patients. Although all HIV strains appear to be capable of infecting normal PHA-stimulated peripheral blood T lymphocytes, many strains show differences in ability to infect macrophages, leukemic T cell lines and other cell types *in vitro*, and these differences might correlate with different pathogenic events *in vivo*.

The main goal of this project is the study of HIV cell tropism *in vitro* and *in vivo* with an aim to understanding the role of different HIV strains in influencing the variations in disease progression and pathogenesis in infected individuals. Several years ago we developed a quantitative *in vitro* infectivity assay for HIV by inserting an expression vector encoding the human CD4 gene into a HeLa cell line. In subsequent years we applied this approach to studies of HIV infection of other types of human and mouse cells expressing CD4 in order to gain some insight into host cell factors which influence susceptibility to HIV. In addition we have generated a new CD4-positive HeLa clone with high sensitivity to patient-derived HIV isolates. This cell line detects T cell tropic HIV strains, and we have used it to map viral determinants of cell tropism as well as to study drug resistance of patient HIV isolates and infectious HIV expression in peripheral blood samples as a function of clinical disease status.

Results on HIV isolates from a variety of patients indicate that specific areas within the V3 loop of the HIV envelope gene are involved in determining tropism for macrophages versus CD4-positive HeLa cells or T lymphocytes. As expected, there was high sequence variability in the V3 regions of T cell tropic viruses from different patients; however, surprising homogeneity of V3 sequences of macrophage-tropic viruses from different patients was observed. Together with the findings of other workers that HIV isolated from patients shortly after infection contains this same macrophage-tropic consensus V3 sequence, these results suggest that macrophage-tropic HIV strains may be the main strains transmitted between patients and, thus, they might be ideal targets for an HIV vaccine.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-854-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00580-04 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemistry of Scrapie Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Other:	Richard Race, D.V.M.	Research Veterinarian	LPVD
	Bruce Chesebro, M.D.	Chief	LPVD

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical IIDEA project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The accumulation in the CNS of an amyloidogenic, protease-resistant isoform (PrP-res) of the host protein PrP is specific to scrapie and related transmissible spongiform encephalopathies (TSEs) and important in the pathogenesis of these neurodegenerative diseases. Our major goals are to determine 1) the mechanism of PrP-res formation in scrapie-infected cells, 2) methods of preventing PrP-res accumulation, and 3) the relationship of PrP-res to the transmissible scrapie agent.

In pursuit of these goals, we have sought inhibitors of PrP-res accumulation. As reported last year, we have used scrapie-infected mouse neuroblastoma cells to identify Congo red, an amyloid stain, and several sulfated glycans as potent and selective inhibitors of the accumulation of PrP-res. We have now compared the inhibitory properties of numerous compounds related to these inhibitors in order to better define the molecular attributes of inhibitors of PrP-res accumulation. The effective inhibitors are either highly sulfated glycosaminoglycans (GAGs) or are GAG analogs and this fact has suggested a mechanism of action for these inhibitors. Since PrP-res is known to contain GAGs (heparin sulfate), as do all other naturally derived amyloids, we have hypothesized that these sulfated inhibitors competitively block an interaction of PrP with an endogenous GAG that is critical for the accumulation. Consistent with this hypothesis, we have observed that 1) heparinase treatment on intact cells inhibits PrP-res accumulation, indicating a role for an endogenous heparin-like GAG in the amyloidogenic process and 2) the normal PrP precursor of PrP-res binds to both heparin-agarose and immobilized Congo red and both interactions can be blocked with free Congo red or sulfated glycan inhibitors of PrP-res accumulation. Interestingly, we have also shown that the elimination of cellular PrP-res by Congo red treatment is accompanied by a loss of scrapie infectivity, suggesting that PrP-res is important in scrapie agent replication. *In vivo* studies of the effects of Congo red on the development of scrapie in mice and hamsters are underway. No dramatic therapeutic effects have been observed, but we suspect that this may in part be due to the inability of Congo red to cross the blood brain barrier. Regardless of the efficacy of Congo red against scrapie *in vivo*, we believe that the potent inhibition of PrP-res accumulation that we have observed *in vitro* suggests that disruption of amyloid-GAG interactions is an attractive therapeutic strategy to consider for many otherwise untreatable amyloidoses, such as Alzheimer's disease.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00611-03 LPVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enrichment of Hematopoietic Stem Cells from Mouse Bone Marrow

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Hematopoiesis is an ongoing developmental process in adult mammals. The details of the early stages of this process remain obscure, mainly because the very low frequency of hematopoietic stem cells (HSC) in bone marrow limits the ability to study these cells. The goal of this project is to define phenotypic characteristics which will help to identify HSC as they normally exist in bone marrow tissue. Further, this project aims to document growth and differentiation characteristics of normal HSC by transplantation studies and by in vitro culture techniques. We are also interested in defining optimal conditions for infecting enriched populations of HSC with retroviral constructs. In addition, this project aims to utilize several approaches to characterize genes expressed during early hematopoiesis. We have established that in the mouse, the cell surface phenotype of HSC varies significantly among different mouse strains in an allele-specific manner. Recently, we demonstrated that bone marrow from mouse strains which do not express the Ly-6A/E (Sca-1) molecule on most HSC contains a low frequency of Ly-6A/E+ HSC. These rare Ly-6A/E+ HSC are functionally very similar to those in the prototype mouse strains where 99% of HSC express the marker. This subset of cells may represent HSC that have recently divided, or are currently contributing to the progenitor pool rather than resting as most HSC normally do. We hope to clarify the significance of this rare HSC subset in further studies. In order to define the functional potential of the quiescent and activated components of the HSC compartment, we are performing transplantation studies using very small numbers of purified HSC to repopulate lethally irradiated animals. These experiments will demonstrate the potential of individual HSC to function over extended periods of time, and we hope to obtain quantitative information on the ability of HSC to self-renew. Our preliminary experiments show significant repopulation of irradiated recipients (50-90% of circulating peripheral blood cells) after transplantation of only 5 purified HSC. To study the expression of cytokine and cytokine receptor genes during HSC differentiation, we have utilized in situ hybridization to identify expression of these genes after transplantation of HSC into irradiated thymic lobes. We hope this model system will allow identification of cytokines that are active during HSC differentiation in vivo and will thus allow us to develop in vitro culture systems that accurately mimic normal HSC development.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-884-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00673-01 LPVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Estrogen Hepatotoxicity and Hepatocarcinogenicity in Hamsters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Other:			
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Estrogen is known to adversely affect hepatic function. Thus biliary function is compromised after administration of exogenous estrogens to normal humans and experimental animals, although this diminished function is minimal and does not result in increased serum bilirubin levels. In certain genetically susceptible women, high estrogen levels are associated with increased serum bilirubin levels, a condition called cholestatic jaundice of pregnancy. Also in humans, estrogen administration (as in oral contraceptives, etc.) is known to be associated with the development of hepatic tumors. The liver of the Armenian hamster is extraordinarily sensitive to estrogens so that this rodent can be used as a novel experimental model to study these two phenomena. The Armenian hamster is unique because after estrogen administration it develops an acute jaundice with serum bilirubin levels 15-20 mg per cent. After chronic estrogen exposure, various benign and malignant neoplastic changes are detected in the liver. Why is the liver of Armenian hamster so extraordinarily sensitive to effects of exogenous estrogen? We are studying hepatocytes isolated in primary culture tissue from Armenian hamsters to look for unusual characteristics when compared to Syrian hamster hepatocytes, a hamster which is not adversely affected by exogenous estrogen.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-884-939

LABORATORY OF VIRAL DISEASES
1993 ANNUAL REPORT
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LABORATORY OF VIRAL Diseases
ANNUAL REPORT, 1993
SUMMARY

The Laboratory of Viral Diseases carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. These studies are designed to increase fundamental knowledge as well as to facilitate the development of new approaches to the prevention and treatment of virus infections. Current topics of basic research include virus entry into cells, regulation of gene expression, mechanisms of DNA replication, assembly and transport of viral proteins and particles, action of virus growth factors, determinants of virus virulence, host resistance genes, and viral targets of humoral and cellular immunity. Applied areas of investigation include the development of recombinant expression vectors, candidate vaccines, and antiviral agents. These studies involve a wide range of DNA and RNA viruses including human immunodeficiency virus.

Regulation of virus gene expression. Viruses provide advantageous systems for studying basic aspects of gene expression and can be used as expression vectors. Several different virus systems are currently under investigation by members of LVD.

Poxviruses. Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. In order to express and replicate their genomes outside of the nucleus, poxviruses must encode enzymes for these processes. This feature makes poxviruses, among which vaccinia virus is the prototype, unique for studying the regulation of transcription. Additional information regarding transcription also is needed to improve vaccinia virus expression vectors, which are currently being evaluated as live vaccines against several diseases including AIDS.

Temporal regulation of vaccinia virus intermediate gene expression. Vaccinia virus was recently found to contain a class of genes, called intermediate, that are expressed after viral DNA replication. The steady-state levels of mRNAs encoded by several intermediate genes were determined and compared to the levels of well characterized early- and late-class genes. After synchronous infection of HeLa cells, the early mRNAs were detected within 20 minutes and peaked at about 100 minutes; the intermediate mRNAs were detected at 100 minutes and peaked at 120 minutes; and the late mRNAs were detected at 140 minutes and increased thereafter. Upon reaching maximum levels, the early and intermediate mRNAs declined at rates consistent with half-lives of 30 minutes or less, providing the basis for rapid changes in gene expression. The structures of intermediate mRNAs were analyzed and were shown to possess 5′ poly(A) leaders evidently produced by slippage of the RNA polymerase following initiation. The construction and analysis of recombinant viruses containing mutated intermediate promoters regulating a reporter gene provided information regarding the core and initiator elements of intermediate promoters that was consistent with previous transfection studies. **(Baldick and Moss)**

Vaccinia virus capping enzyme also serves as an intermediate stage transcription factor. At least three components are required for *in vitro* transcription of intermediate class genes: the viral RNA polymerase, a partially purified transcription factor, and the viral capping enzyme. By transcribing different length truncated templates, we were able to show that the transcription factor activity of the capping enzyme is separate from its role in modifying the $5'$ end of the mRNA. **(Harris, Rosales and Moss)**

Evidence that the product of the intermediate A1L gene is a late transcription factor. Previous transfection studies had identified three intermediate-stage genes (G8R, A1L, and A2L) that are required for transactivation of late gene expression. The product of G8R gene was shown to function as a transcription factor *in vitro.* The amount of the 17 kD product of the A1L gene present in infected cells was too low to purify. Therefore, the gene was overexpressed in mammalian and insect cells. The recombinant protein was extensively purified and shown to stimulate transcription of templates containing late promoters *in vitro.* **(Keck, Kovacs and Moss)**

Construction and characterization of a conditional lethal mutant of vaccinia virus with an inducer-dependent late transcription factor. A recombinant vaccinia virus that has the G8R gene under *Escherichia coli lac* operator control was constructed. In the absence of inducer, synthesis of the G8R protein was inhibited by more than 99%, synthesis of early and intermediate mRNAs was unaffected, intermediate proteins accumulated to higher-than-normal levels, synthesis of late mRNA and protein was reduced by 90%, viral DNA was replicated but concatemeric molecules accumulated, not even the earliest stages of virion assembly were detected, and virus yield was reduced by 10^{-3} to 10^{-4}. **(Zhang and Moss)**

The small subunit of the vaccinia virus poly(A) polymerase stimulates the rate of adenylate addition. The vaccinia virus-encoded poly(A) polymerase consists of two subunits. The 55 kD large subunit can add a maximum of 35 adenylates to the $3'$ end of an RNA in a rapid and processive manner. The overexpressed and purified 39 kD small subunit had no catalytic activity of its own but stimulated the large subunit to make long poly(A) chains. The role of the small subunit is to increase the affinity of the poly(A) polymerase for the growing poly(A) tail. **(Gershon and Moss)**

Virus DNA replication. Viruses are useful systems for analyzing the diversity of mechanisms employed in DNA replication. In addition, the virus-encoded factors provide potential targets for chemotherapy.

Herpesviruses. Several members of the herpesvirus family are significant human pathogens. These include herpes simplex virus (HSV), the best-studied of the family, which causes painful skin lesions and occasionally causes life-threatening encephalitis, and Epstein-Barr virus (EBV), which causes infectious mononucleosis and is thought to be involved in some lymphocytic cancers. The study of herpesvirus DNA synthesis is useful as a model for eukaryotic DNA replication and for designing new antiviral strategies. Previous work from this laboratory demonstrated that seven HSV genes are necessary and sufficient for authentic origin-dependent DNA replication. Current efforts are directed toward studying this process with purified proteins, with identifying required host proteins, and with extending our studies to the lytic replication of EBV.

Reconstitution of DNA synthesis in vitro. To gain further insight into how the required HSV replication proteins interact at a replication fork, we have reconstituted rolling circle DNA replication *in vitro* using purified HSV replication proteins acting on a primer-template having the properties of a preformed replication fork. Our results suggest that *1)* UL8 is required for leading strand synthesis as well as for efficient utilization of primers during lagging strand synthesis; *2)* even when UL8 is present, lagging strand synthesis is very inefficient, suggesting there may a critical cellular factor missing from our system; and *3)* the cellular protein RP-A simulates DNA synthesis in this system. **(Gottlieb and Challberg)**

Mutational analysis of the components of the HSV-1 helicase-primase complex. We have undertaken mutational analysis of both UL8 and UL52 in an attempt to understand the functional contribution of these polypeptides to the helicase-primase complex. Base substitution mutations were introduced into five regions of the UL52 gene that are highly conserved among HSV and the related herpesviruses HCMV, EBV, and VZV. These mutants were analyzed by an *in vivo* complementation assay, and it was found that at least one mutation in each conserved region failed to support DNA replication. One of these mutants has been analyzed in detail. This mutant, in which amino acid 628 is changed from asp to gln, had equivalent levels of helicase activity compared to the wild-type UL5/UL8/52 complex, supported leading strand synthesis in the reconstituted fork system, but was devoid of primase activity. **(Klinedinst, Fabisch, Sherman, and Challberg)**

Studies on the polymerase processivity subunit UL42. The HSV-1 DNA polymerase consists of a heterodimer of the UL30 (Pol; the catalytic subunit) and UL42 polypeptides. Our previous data have shown that the role of UL42 in the DNA polymerase complex is to act as a "clamp," decreasing the probability that the polymerase will dissociate from the template after each cycle of catalysis. Studies of mutated derivatives of UL42 have shown that the carboxyl-terminal third of the polymerase accessory factor, UL42, is not essential for the function of the protein in DNA replication and have provided additional support for the sliding clamp model for UL42 function. In addition, we have found somewhat unexpectedly that UL42 does not increase the fidelity of DNA synthesis. **(Gottlieb, Lantz, Zhen, and Challberg)**

Characterization of the origin binding protein. We have previously shown that the protein encoded by the HSV UL9 gene has two functions characteristic of replication initiator proteins: it binds specifically to the origins of replication, and it has an intrinsic helicase activity. The helicase activity was shown to be stimulated by the single-stranded DNA binding protein ICP8, and direct binding measurements have verified the existence of an interaction between the two proteins. A truncated derivative of UL9 comprising the carboxyl-terminal 317 amino acids retains the origin binding activity of the full-length protein and retains specific interaction with ICP8 as well. In contrast to the full-length protein, however, the truncated derivative exists as a monomer rather than a dimer in solution and fails to link the two binding sites at the origin by protein-protein interaction. **(Fierer and Challberg)**

Analysis of the role of host proteins in HSV DNA replication. Indirect experimental evidence has suggested that host cell proteins might be required for HSV DNA replication. We have started to develop an assay for host protein involvement that is based on microinjection of monoclonal antibodies raised against known cellular protein. We are initially concentrating on the cellular single-stranded DNA binding protein (called RP-A), but the assay could be extended to any host protein for which there is an inhibitory monoclonal antibody. We are now well on the way toward overcoming the technical hurdles inherent in this approach. **(Lantz, Challberg)**

Epstein-Barr lytic DNA replication. Analysis of the structure of the EBV lytic origin and EBV genes required for lytic DNA replication suggests that while the mechanism of DNA synthesis at an established replication fork is very similar to what occurs with HSV, the mechanism of initiation is likely to be very different. In particular, initiation depends not on a specialized viral initiator protein analogous to UL9, but on a key transcriptional transactivator protein (BZLF1). In this respect, initiation of EBV lytic DNA replication may be more similar to the initiation of cellular chromosomal DNA replication. We have begun a biochemical analysis of EBV DNA replication. At present, we are in the process of constructing the key reagents necessary for these studies, which consist primarily of recombinant baculoviruses expressing the known EBV-encoded replication proteins. **(Zhen, Challberg)**

Poxviruses. Poxviruses provide a unique experimental system, since the enzymes and factors are encoded within the virus genome and replication occurs in the cytoplasm. Previous studies revealed that vaccinia virus DNA is replicated as concatemeric structures which are resolved into unit-length genomes with hairpin ends.

Mutagenesis of a conserved region of vaccinia virus DNA polymerase. Vaccinia viruses containing codon alterations in a conserved region of the vaccinia virus DNA polymerase gene have been selected by linkage to the aphidicolin resistance mutation. Three different viruses with substitutions for the consecutive valine tyrosine residues in the conserved region have been isolated. The vaccinia virus plaque assay indicates what substitutions in the conserved region can be sustained by the virus *in vivo*. To determine the effects of these and other substitutions on enzyme activity, current efforts are directed at expressing the vaccinia enzyme in *E. coli*. For this purpose, methods have been improved for copying the vaccinia gene and for testing for its presence in bacteria. **(DeFilippes)**

Identification and characterization of the vaccinia virus gene encoding the nicking-joining enzyme. A nuclease has been detected in virions which converts plasmids containing the vaccinia virus concatemer junction into nicked circular, linear, and cross-linked linear molecules by a region specific cleavage near the border of the hairpin loop. We have isolated the predominant protein that chromatographs with these activities and identified the product as corresponding to the open reading frame A5L, a late gene of approximately 33 kD. Presently, efforts are being made to determine if A5L is the sole factor for high levels of nuclease activity and if the gene is essential for virus growth. **(Merchlinsky)**

Identification of proteins involved in telomere resolution. A search of the vaccinia virus Copenhagen strain viral genome by Ken Abremski using an algorithm he developed to detect members of the integrase family of site-specific recombination proteins detected one open reading frame in vaccinia, G9R, as a candidate for an integrase. The protein encoded by this open reading frame has not been previously studied, has a molecular weight of approximately 39 kD, and did not serve as a telomere resolvase when expressed in plasmid constructs under control of the T7 promoter and monitored for the resolution of cotransfected plasmids. Current efforts are designed to determine if G9R participates in telomere resolution by purification and characterization of the protein and the design of viral genomes that conditionally express G9R so that we can investigate the phenotype of the viral infection in the absence of G9R. **(Merchlinsky)**

Determination of DNA helicase involved in vaccinia virus DNA replication. A search of the viral genome has revealed a series of helicase domains in vaccinia virus open reading frames. Three of the open reading frames, A18R, A32R, and D5R, have unknown functions in vaccinia virus. Two of the products, A18R and D5R, have been shown to be synthesized early, consistent with a role in DNA replication, and in one, D5R, a conditionally lethal mutation has been isolated that confers a DNA replication-defective phenotype at elevated temperatures. We have cloned the open reading frames for all three genes, and these proteins will be tested for DNA-dependent directional helicase activity consistent with participation at the fork of viral DNA synthesis. **(Merchlinsky)**

Virus structure, entry, assembly, and dissemination. Information regarding the mechanisms of virus structure, entry, assembly, and dissemination may provide important insights into antigenic structure for vaccines and targets for therapy.

Human immunodeficiency virus (HIV). HIV is the etiological agent of acquired immune deficiency syndrome (AIDS). At present, there is no effective vaccine against this virus, and therapeutic agents provide only limited help. The envelope protein is the best target for neutralizing antibody and is therefore of particular importance for development of a vaccine.

Novel assay for CD4/env-mediated cell fusion. HIV infection of human T lymphocytes or macrophages is initiated by binding of the external subunit (gp120) of HIV env to CD4 molecules on target cells; direct fusion between the viral and cell membranes then ensues by poorly understood mechanisms. Our experimental approaches to the fusion problem rely extensively on recombinant vaccinia vectors to transiently express CD4 and env on separate cell populations. We have developed a novel fusion assay in which one cell population also expresses vaccinia-encoded bacteriophage T7 RNA polymerase, and the other contains the *E. coli lacZ* gene linked to the bacteriophage T7 promoter. Upon mixing the two populations, cell fusion results in activation of the *lacZ* gene and accumulation of β-galactosidase in the fused cells (measured by enzymatic assay of cell lysates or by *in situ* staining). Fusion-dependent enzyme activity is detected within one hour after cell mixing; sensitivity is greatly enhanced compared to assays based on quantitation of syncytia. The assay provides great advantages for mechanistic studies of fusion and for screening fusion-blocking antibodies and drugs. **(Nussbaum, Broder, and Berger)**

Use of vaccinia vectors in primary human macrophages. Application of the vaccinia system to study HIV env-mediated fusion in primary macrophages requires characterization of vaccinia infection in these cells. We observed that macrophages support early, but not late, events of the vaccinia cycle. Experiments with reporter genes indicated that expression can be obtained in these cells from early and intermediate, but not late, vaccinia promoters; the hybrid T7 RNA polymerase/T7 promoter system is also functional, indicating the utility of the above-described reporter gene activation fusion assay. Using the appropriate promoters, expression of CD4 and HIV env in primary macrophages was demonstrated. **(Broder, Kennedy, and Berger)**

HIV-1 tropism. Individual isolates of HIV-1 vary markedly in their ability to infect different $CD4^+$ cell types: some productively infect T cell lines but not primary macrophages, whereas others show the opposite specificity. The determinants for this tropism reside within the env glycoproteins. To determine the basis for this specificity, we prepared a battery of vaccinia-based env constructs from HIV-1 isolates with different cell-type tropisms and used a newly developed assay to measure their ability to mediate cell fusion with different target cells. Our results provided the first direct evidence that the macrophage *vs.* T cell line tropisms of different HIV-1 isolates are associated with highly selective fusogenic properties of the corresponding envelope glycoproteins. **(Broder and Berger)**

Accessory cellular factors in env/CD4-mediated fusion. The human CD4 molecule is able to support HIV-1 env-mediated fusion when present on the surface of a variety of human cell types; however, fusion does not occur when the same CD4 molecule is expressed on non-human cells. We have used the newly developed reporter gene activation assay to confirm our previous finding that animal cells expressing human CD4 can be rendered fusion-competent by formation of transient hybrids with human cells, suggesting the presence of a human-specific accessory fusion factor(s). Preliminary results indicate that this component can be transferred to animal cells by PEG-mediated fusion of membrane vesicles derived from human cells. **(Nussbaum, Broder, and Berger)**

Enhancement of CD4/env-mediated fusion by co-expression of furin. It has been reported that co-expression of furin (a subtilisin-like protease) enhances processing of glycoproteins of several enveloped viruses, including HIV. Using the vaccinia system, we tested the effects of co-expression of HIV env and furin; fusion activity was found to be greatly enhanced for envs of both T cell line-tropic and macrophage-tropic isolates. This finding has considerable practical utility in the analysis of fusion mechanisms. **(Broder, Nussbaum, and Berger)**

Mutational analysis of the assembly domain of the HIV-1 envelope glycoprotein. The amino terminal 129 amino acids of gp41 of the human immunodeficiency virus type 1 (HIV-1) envelope (env) glycoprotein constitute the assembly domain required for efficient oligomer formation and stability. Point mutations in highly conserved structural features including cysteine residues, potential N-linked glycosylation sites, and a leucine zipper motif have been made in a soluble secreted form of env (env_{sec}). No single-point mutation had adverse effects on env protein oligomerization; however, truncation of the C-terminus of gp41 from 129 amino acids to 68 amino acids drastically reduced oligomezation efficiency, indicating that amino acids 68-129 are essential for assembly. **(Earl, Moss)**

Monoclonl antibodies to oligomeric forms of the HIV-1 envelope protein. A recombinant vaccinia virus encoding a secreted oligomeric form of the HIV-1 envelope protein, lacking the transmembrane domain and site of cleavage between gpl20 and gp41, was engineered. Purified dimers and tetramers were used to inoculate mice and hybridomas that produce monoclonal antibodies with specific reactivity to oligomeric forms of the envelope proein. Efforts to map the sites of antibody binding and determine their virus neutralizing properties are in progress. **(Earl, Broder, and Moss)**

***Influenza virus*.** The major envelope glycoprotein of influenza virus, the hemagglutinin (HA), is the most extensively characterized viral envelope protein and has proven to be a useful model for similar proteins present on other viruses. Influenza remains a significant human pathogen, causing more mortality in the U.S. than any other virus. Since protection against influenza is provided nearly exclusively by the antibody response to the HA, it is essential to understand its structure to improve existing vaccines, which are only marginally effective.

Folding, assembly, and transport of viral glycoproteins. Monoclonal antibodies (mAbs) that distinguish between monomeric and trimeric forms of HA were used to localize the site of HA assembly in influenza virus-infected cells. This was achieved using a laser confocal scanning microscope to localize fluorescent-tagged antibodies or an electron microscope to localize colloidal gold-tagged antibodies on ultrathin cryosections. Preliminary results suggest that trimerization occurs only after the HA has been exported from the ER. This finding is important, since cell biology dogma asserts that assembly of multimeric proteins is required for their export from the ER. **(Yewdell, Bennink)**

Mechanism of brefeldin A action. Brefeldin A (BFA) is a fungal metabolite widely used by cell biologists and immunologists due to its unique ability to interfere with vesicular trafficking. To better understand how it works, fluorescent analogs were synthesized and localized in living or fixed cells by laser confocal scanning microscopy. The BFA conjugates localized to the endoplasmic reticulum and Golgi complex of living and fixed cells, which makes them valuable as probes to localize these organelles. Although the analogs maintained biological activity, this required cleavage by cellular esterases to release BFA from the conjugate. Nonetheless, inasmuch as the pattern localization of the BFA conjugates is unusual (and differs from the fluorescent probes contained in the conjugate), this suggests that the action of BFA is related to its specific perturbation of these membranes. **(Deng, Bennink, and Yewdell)**

Confocal microscopy of cells. The laser confocal scanning microscope (LCSM) provides unparalleled resolution in three dimensions for localizing fluorescent probes in cells. In conjunction with powerful minicomputers and special software, it is possible to crease accurate 3-D reconstructions of the distribution of the fluorescent probe. The LCSM is being used to determine the intracellular trafficking of defined molecules, the subcellular localization of newly discovered cellular and viral genes, the localization of novel dyes, and the migration of developing lymphocytes. **(Yewdell, Bennink)**

Assembly of vaccinia virus: role of the intermediate compartment between the endoplasmic reticulum and the Golgi stacks. The assembly pathway of vaccinia virus displays several unique features, such as the production of two structurally distinct, infectious forms. One of these, termed intracellular naked virus (INV), remains cell-associated, while the other, termed extracellular enveloped virus (EEV), is released from the cell. In addition, it has long been believed that INVs acquire their lipid envelopes by a unique example of *de novo* membrane biogenesis. To examine the structure and assembly of vaccinia virus, we have used immunoelectron microscopy using antibodies to proteins of different subcellular compartments as well as a phospholipid analysis of purified INV and EEV. Our data are not consistent with the *de novo* model of viral membrane synthesis, but rather argue that the vaccinia virus DNA becomes enwrapped by a membrane cisterna derived from the intermediate compartment between the ER and the Golgi stacks, thus acquiring two membranes in one step. Phospholipid analysis of purified INV supports its derivation from an early biosynthetic compartment. This unique assembly process is repeated once more when the INV becomes enwrapped by an additional membrane cisterna, in agreement with earlier reports. The available data suggest that after fusion between the outer envelope and the plasma membrane, mature EEV is released from the cell. **(Griffiths, Doms, and Moss)**

Release of progeny vaccinia virus from the cell membrane is regulated by a viral envelope glycoprotein encoded by the A34R gene. Vaccinia virus strains vary considerably in the amounts of extracellular enveloped virus (EEV) that they release from infected cells. The IHD-J strain produces up to 40 times more EEV than does the related WR strain and consequently generates elongated comet-shaped virus plaques instead of sharply defined round ones in susceptible monolayer cells under liquid meduim. The difference in EEV formation is due to the retention of enveloped WR virions on the cell surface. By using WR and

IHD-J DNA fragments for marker transfer and analyzing the progeny virus by the comet formation assay, we determined that gene A34R and at least one other gene regulate the release of cell-associated virions. Gene A34R encodes an EEV-specific glycoprotein with homology to C-type animal lectins. One codon mutation (Lys-151→Gly), which is located in the putative carbohydrate recognition domain, was sufficient to transfer a comet-forming phenotype to WR virus. These data indicate that the A34R-encoded glycoprotein is involved, through its lectin homology domain, in the retention of progeny virus on the surface of parental cells and raise the possibility that the protein also has a role in virus attachment to uninfected cells. **(Blasco, Moss)**

Characterization of a 42 kD class I membrane component of the extracellular vaccinia virus envelope. Using a reverse genetic approach, we have demonstrsted that the product of the B5R open reading frame (ORF), which has homology with members of the family of complement control proteins, is a 42 kD membrane glycoprotein present in the extracellular enveloped (EEV) form of vaccinia virus but absent from the intracellular naked (INV) form. Metabolic labeling with [^{3}H]glucosamine and [^{3}H]palmitate revealed that the B5R product is glycosylated and acylated. The C-terminal transmembrane domain of the protein was identified by constructing a recombinant vaccinia virus that overexpressed a truncated, secreted form of the B5R ORF product. By N-terminal sequence analysis of this secreted protein, the site of signal peptide cleavage of gp42 was determined. Immunogold labeling of EEV and INV with a specific monoclonal antibody demonstrated that the protein was found exclusively on the EEV membrane. Deletion of the B5R gene prevented the formation of the extracellular virus envelope and virus dissemination. **(Wolffe, Moss)**

Glutaredoxin homolog encoded by vaccinia virus is a virion-associated enzyme with thioltransferase activity. Glutaredoxins (GRXs), also known as thioltransferases, use glutathione as a cofactor for reduction of disulfides in prokaryotes and eukaryotes. We demonstrated that the vaccinia virus 02L open reading frame encodes a functional GRX, as predicted from sequence homology. The 12 kD protein product of the 02L open reading frame was synthesized after viral DNA replication, coincident with a major increase in cytoplasmic glutathione-dependent thioltransferase activity. The protein was associated with purified vaccinia virions and was not released by treatment with a nonionic detergent unless dithiothreitol was added. The virion-derived protein, as well as a recombinant form expressed in *Escherichia coli*, exhibited thioltransferase and dehydroascorbate reductase activities indicative of a functional GRX. The postreplicative synthesis of vaccinia virus GRX and its association with virions suggest that the enzyme may have novel roles in the virus growth cycle. **(Ahn, Moss)**

Viral immunology. Class I molecules of the major histocompatibility complex class (MHC) consist of a highly polymorphic heavy chain complexed to B_2-macroglobulin. Class I molecules are expressed on virtually all cell types. Their sole function is to bind antigens and present them to T cells bearing CD8 molecules. These T cells are known as cytotoxic T lymphocytes (CTLs) due to their ability to lyse histocompatible cells in an antigen-specific manner. CTLs play a critical role in eradicating intracellular pathogens and tumors. On the negative side, they are involved in organ rejection and in many autoimmune dyscrasia.

Structure and function of antigenic peptide transporters. The efficient presentation of antigens to the immune system requires the expression of a transporter that conveys peptides from the cytosol to the endoplasmic reticulum (ER). Functional experiments demonstrated that transporters from different species facilitated presentation of the same set of peptides, suggesting that the limited polymorphism present in transporter genes in human genes goes not play a major role in governing immune responsiveness. Production of vaccinia virus recombinants expressing the subunits of the transporter allowed for the immunocytochemical localization of the transporter to the ER and for biochemical characterization. **(Russ, Bennink, and Yewdell)**

Class I molecule assembly and trafficking. Class I molecules of the major histocompatibility complex (MHC) bind peptide antigens derived from cytosolic proteins and carry them to the cell surface for recognition by T cells. We genetically altered a class I molecule to be retained in the endoplasmic reticulum (ER) following its synthesis, and used this molecule to demonstrate that peptide binding to class I molecules occurs in the ER. Using a fluorescent antigenic peptide, we demonstrated first that peptide containing class I molecules present at the cell surface are delivered to lysosomes and, second, that extracellular peptides are somehow transported to class I molecules present in the ER. We also obtained evidence that peptide-free class I molecules exported from the ER are destroyed in the Golgi complex. **(Day, Deng, Bennink, and Yewdell)**

Antigen Processing in Lower Eukaryotic Cells. To better define the specialized components of the antigen processing machinery we are attempting to reconstitute antigen processing in insect cells, which do not have a major histocompatibility complex, (MHC) and would not be expected to have any of the specialized components needed to produce antigenic peptides and transport them to class I MHC molecules. We found that following infection of mosquito cells with vaccinia viruses gene products under the control of early transcriptional promoters are expressed at reasonably high levels. Using this system we could demonstrate that class I molecules properly fold and assemble in insect cells and maintain the capacity to bind antigenic peptides. We are currently determining whether insect cells can produce peptides from biosynthesized proteins and deliver them to class I molecules. **(Deng, Bennink, Yewdell)**

Antigen presentation by human tumors. It has long been hoped that tumor specific T cells culd be used to prevent and treat cancers. With this goal in mind we created a novel type of vaccine that bypasses the normal antigen processing machinery by directly delivering antigenic peptides to the endoplasmic reticulum, the site of peptide association with major histocompatibility compl x class I molecules. We are currently testing the ability of these vaccines to induce tumor specific T cells and prevent and cure tumors in mice. **(Bennink, Yewdell)**

Proteolytic generation of antigenic peptides. One of the deepest mysteries of antigen presentation is how antigenic peptides are generated by cells. We found that two genes suspected to play a role in peptide generation are not required for efficient antigen presentation. We have created and characterized a number of recombinant vaccinia viruses that will enable to study in detail the role of ubiquitin-targeted protein degradation in antigen presentation. We also initiated studies to determine whether some cellular proteins are selectively spared from the antigen processing machinery **(Cox, Anton, Yewdell, Bennink)**

Delivery of antigens to the MHC class I processing pathway. In an attempt to devise a non-infectious vaccine for eliciting T cell responses, we studied whether folic acid could target extracellular proteins to the MHC class I antigen processing pathway. Our findings suggests that this is not a useful vaccine strategy. As an alternative strategy for designing vaccines with enhanced ability to elicit T cell responses, we produced recombinant vaccinia viruses expressing proteins that do not require specialized antigen processing machinery. Preliminary results suggests that these recombinant viruses possess enhanced immunogenicity. (**Anderson, Yewdell, Bennink**)

Proteolytic processing of antigenic peptides in the endoplasmic reticulum. A critical question in antigen processing is whether antigenic peptides are trimmed to their final length after they have been imported into the endoplasmic reticulum (ER) from the cytosol. To explore this question, we created a number of vaccinia virus recombinants that express proteins targeted to the ER that consist of antigenic peptides surrounded by different flanking sequences. Using cells dependent on ER processing for antigen presentation, we found that the ER is able to liberate antigenic peptides from short flanking sequences but not from very large flanking sequences. These findings indicate that the ER has at least some endogenous peptide trimming activity (**Link, Yewdell, Bennink**).

Virus Pathogenesis. Recent advances in recombinant DNA technoloy, cell biology and immunology have provided an unparalleled opportunity to study the fine detail of the virus and host genes involved in virus pathogenesis. The study of vaccinia virus (VV) and cowpox virus (CPV) in the rabbit or chick embryo, and especially ectromelia virus (EV) in the mouse have a number of advantages over other virus/animal models. For example with ectromelia virus infections of the mouse: 1) there are disease-resistant and -susceptible strains of mice available; 2) the natural route of infection is known and can be mimicked by the foot-pad route; 3) the natural history of the disease (mousepox) is well understood; and 4) experimental virus transmission between an index mouse and contacts is reproducibly attainable in the laboratory.

In/Vivo Replication Mutant. An EV ORF predicted to encode a p28 protein was sequenced and found to contain a zinc finger motif that is found in a select group of proteins, some of which have transcriptional functions. An intact p28 gene was also present in CPV, but not in VV. In vitro experiments showed the EV p28 protein to bind Zn^{+2}, which apparently mediated a nonspecific interaction with certain DNA sequences. Preliminary data suggest the protein is expressed at low levels in EV-infected cells and, when overexpresssed *via* VV T7 vectors, is found in the nucleus. An EV mutant lacking this ORF was shown to replicate as efficiently as WT in L_{929}, primary mouse embryo, and primary mouse ovarian cells; however, this mutant virus replicated at least 100-fold less efficiently than EV WT in tested tissues of the nude, SCID, or intact mouse, spread more slowly from the primary site of inoculation to internal organs, and had an LD_{50} that was 1000 times higher than the WT virus. (**Koonina, Buller**)

Nitric Oxide is a Potent Antiviral Metabolite. Interferon-γ is important for the recovery of mice from infection with EV. An IFN-γ-induced antiviral mechanism was observed in primary or tissue-culture macrophages, but not epithelia or fibroblastic cell lines. The antiviral activity induced in macrophage cultures was blocked by inhibitors of nitric oxide synthase. Virus replication was also diminished in epithelial cells transfected with cDNA encoding the inducible nitric oxide synthase or treated with organic compounds that generate nitric oxide. In mice, an inhibitor of nitric oxide synthase converted sublethal, resolving EV infections into fulminant mousepox. Thus, induction of nitric oxide synthase was necessary and sufficient for a substantial antiviral effect of IFN-γ *in vitro* and *in vivo.* **(Karupiah, Buller)**

Regulation of Complement Activity by Vaccinia Virus Complement-Control Protein. A major protein secreted by vaccinia virus-infected cells has structural similarity to the superfamily of complement-control proteins. This vaccinia complement-control protein (VCP) was studied to determine how it regulates complement activation. VCP was bound by C4b and C3b and served as a cofactor with factor I in cleaving these two molecules. VCP inhibited the formation and accelerated the decay of the classical C3 convertase. It also accelerated decay of the alternative pathway convertase, although higher concentrations were apparently needed. In vitro, therefore, VCP interfered with the classical and alternative complement pathways at several steps. In vivo, this interference may increase the virulence of vaccinia virus by enabling it to escape attack by the host's complement system. **(Isaacs, Moss)**

Nuclear localization of an interferon-defense molecule encoded by vaccinia virus. The E3L gene encodes a double-stranded RNA binding protein that may play a role in the defense of vaccinia virus against the effects of interferon. A monoclonal antibody to product of the E3L gene revealed that the 25-kDa protein localized to the nucleus of infected cells. Transfection studies indicated that no other viral proteins were required for this localization. Since the interferon defense activity would likely occur in the cytoplasm, these data suggest that the viral protein may have additional roles in the infectious cycle. **(Yuwen, Cox, Bennink, Yewdell, Moss)**

Virus Expression Vectors. Use of expression vectors has become an important part of recombinant DNA technoloy. Vaccinia virus is now widely used for expression of proteins in mammalian cells and has proven particularly useful for determining the targets of humoral and cellular immunity. Recombinant vaccinia virus is currently being tested as a candidate AIDS vaccine in humans and a rabies vaccine in wild-life.

Nonreplicating vaccinia vector efficiently expresses recomblnant genes. Modified vaccinia Ankara (MVA), a highly attenuated vaccinia virus strain that has been safely tested in humans, was elevated for use as an expression vector. MVA has multiple genomic deletions and is severely host cell restricted: it grows well in avian cells but is unable to multiply in human and most other mammalian cells tested. Nevertheless, we found that replication of viral DNA appeared normal and that both early and late viral proteins were synthesized in human cells. Proteolytic processing of viral structural proteins was inhibited, however, and only immature virus particles were detected by electron microscopy. We constructed an insertion plasmid with the *Escherichia coli lacZ* gene under the control of the vaccinia virus late promoter P11, flanked by sequences of MVA DNA, to allow homologous recombination at the site of a naturally occurring 3500-base-pair deletion within the MVA genome. MVA recombinants were isolated and propagated in permissive avian cells and shown to express the enzyme β-galactosidase upon infection of nonpermissive human cells. The amount of enzyme made was similar to that produced by a recombinant of vaccinia virus strain Western Reserve, which also had the *lacZ* gene under control of the P11 promoter, but multiplied to high titers. Since recombinant gene expression is unimpaired in nonpermissive human cells, MVA may serve as a highly efficient and exceptionally safe vector. **(Sutter, Moss)**

Molecular construction of chimeric vaccinia virus genomes by ligation of DNA: recombination independent production of viral genomes. A new method for production of viral genomes containing insert DNA which relies on ligation of the insert DNA to subgenomic pieces of the vaccinia DNA genome, the transfection of the ligation mix into cells infected with the conditionally defective virus, and the isolation of the genomes containing inserts by plaque assay was developed. Vaccinia virus vectors were designed with a site for the infrequent cutting restriction enzyme NotI positioned so that colormetric detection or resistance to bromodeoxyuridine could be used to identify genomes containing insert DNA. This protocol generates genomes containing insert DNA more than one hundred times as efficiently as by recombination and was independent of size as a 26,000 base pair fragment was cloned as efficiently as a 4,300 base pair fragment. **(Merchlinsky)**

Development of Antiviral Agents. New molecular approaches are being developed for the prevention and treatment of viral diseases. These include recombinant subunit and live vaccines, targeted therapeutics, and novel ways of providing intracellular immunity.

CD4-Pseudomonas exotoxin - candidate HIV therapeutic. A genetically engineered hybrid toxin CD4-PE40, composed of a region of CD4 containing the HIV envelope glycoprotein binding site linked to active domains of *Pseudomonas* exotoxin A, was produced as a collaborative project between NIAID and NCI. The hybrid toxin selectively kills HIV-infected cells and is active against laboratory-adapted and primary HIV isolates. CD4-PE40 has been licensed for clinical development by the Upjohn pharmaceutical company. Phase I clinical trials began August 91 at NIH, and a multicenter expanded Phase I trial was initiated in 1992 by the AIDS Clinical Trials Group for this year. Initial clinical studies suggested unexpected high levels of liver toxicity in HIV-infected patients. Preliminary studies suggest that soluble gp120 enhances the toxicity of CD4-PE40 for hepatocyte cell lines, possibly by enhancing toxin entry via the asialoglycoprotein receptor. The clinical implications of these findings are being pursued. A clinical protocol has also been developed for the use of CD4-PE40 for *ex vivo* treatment of patient PBLs in the context of gene therapy for HIV infection.
(Moss and Berger)

Administrative Changes. Dr. James Rose, Chief of the Molecular Structure Section, retired after 30 years in the Commissioned Corps. Dr. Rose is well known for his pioneering work on the structure and replication of the genome of the AAV virus. Dr. Jonathan Yewdell was appointed head of the newly organized Cell Biology Section. Dr. Thomas Kristie was recruited as a tenure track candidate. He earned a Ph.D. from the University of Chicago under the guidance of Professor Bernard Roizman and received postdoctoral training at MIT with Professor Philip Sharp. Dr. Kristie will carry out a research program on the regulation of gene expression using herpesvirus as a model system.

Honors, Awards, and Service. The Laboratory of Viral Diseases was selected once again by the World Health Organization as a Collaborative Center for Resarch on Virus Vectors with Bernard Moss as head. Moss also serves on a WHO Technical Committee. Moss was elected president of the American Society for Virology; during the coming year, he will serve as the president elect and the following year as president.

Members of LVD serve as editors or editorial board members of numerous scientific publications. Jack Bennink is an Associate Editor of the Journal of Immunology. Mark Challberg is on the editorial board of the Journal of Virology. Moss is an Editor of Virology and serves on the editorial boards of the Journal of Virology, AIDS Research and Human Retroviruses, Advances in Virus Research and the NIH Catalyst.

Members of LVD accepted numerous invitations to visit universities and present major lectures or chair workshops at international and national meetings including the International Congress of Virology, Annual Meeting of the American Association of Immunologists, Interntional Workshop on Herpesviruses, Human Frontier Science Program, the Juan March Institute of Spain, Keystone Conferences and Gordon Conferences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00123-27 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Structure and Replication of Poxvirus DNA

PRINCIPAL INVESTIGATOR	M. J. Merchlinsky	Microbiologist	LVD, NIAID
Others:	B. Moss	Laboratory Chief	LVD, NIAID
	B. Ryan	Technician	LVD, NIAID
	F. Koczot	Technician	LVD, NIAID

COOPERATING UNITS *(If any)*

None

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Poxviruses provide a unique system for studying the replication of DNA. Required enzymes and factors are encoded within the viral genome, and DNA synthesis and processing occurs within the cytoplasmic compartment of the cell. Therefore, it has been possible to apply genetic and biochemical approaches to the study of DNA replication. Our effort has been toward ascertaining the structure and mode of replication of the poxvirus genome, with particular emphasis placed on understanding the processing of the replicative intermediates—including the telomere-like hairpin structure and the enzymes involved in its replication. The replication of vaccinia virus proceeds through concatemeric intermediates which are resolved into unit-length DNA. Mutational analysis has demonstrated that a specific *cis*-acting DNA sequence—highly conserved among poxviruses—as well as the palindromic structure of the concatemer junction, is essential for resolution and that resolution occurred by conservative strand exchange. A model for resolution involving site-specific recombination and orientated branch migration is consistent with these data. We have identified proteins that are candidates for *trans*-acting components of telomere resolution and are characterizing their properties. A procedure has been developed for the construction of viral genomes containing large inserts of foreign DNA by ligation of subgenomic viral DNA fragments in the presence of the insert DNA. These DNA molecules are transfected into cells, where they can be packaged into viral particles and subsequently propagated as virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00126-20 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Functional Analyses of Vaccinia Virus DNA

PRINCIPAL INVESTIGATOR F. M. DeFilippes Research Physicist LVD, NIAID

Others:

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Previous experiments were designed to test the assumption that the conserved residues (CR) of a protein are essential for its function. Procedures were developed for efficient use of the polymerase chain reaction (PCR) to introduce alterations into codons for CRs in a region of the vaccinia virus DNA polymerase. These alterations are linked to a mutation in an adjacent site which confers resistance to aphidicolin (AP). The most significant substitutions were the replacement of a pair of amino acids, tyrosine and valine, by either tryptophan and threonine or trytophan and alanine in the same sequence. Vaccinia virus with these alterations produced normal virus. Attempts to replace the same tyrosine with aspartic acid failed, indicating that a negative charge could not be introduced in this region. Also, attempts to replace the conserved lysine were not successful. To test these *in vivo* changes, I have tried to develop a bacterial cloning and expression system. To obtain more faithful PCR replication of target DNA, I have replaced Taq polymerase with Vent polymerase and modified the reaction conditions. To facilitate cloning procedures, I developed a PCR assay which replaces the "mini prep" procedure for detecting the presence and orientation of inserted DNA. Finally, I modified the affinity resin binding procedure used with pET vectors so that it could be used with detergent-solubilized proteins. Using these procedures, I tried to express a fusion protein which included the carboxy terminal portion of the DNA polymerase. Use of the pMal vector to produce a fusion with the target led to degradation of the fused protein. A pET vector produces a protein of the predicted size in amounts that can be detected by stained gels. If the protein is genuine and can be renatured, it should be suitable for *in vitro* studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00294-11 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Adenovirus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P1:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID

COOPERATING UNITS *(if any)*

R. McPherson, M.D., Department of Pathology, Scripps Clinic, San Diego, CA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.2	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Among objectives of these studies has been the application of physical, biochemical and biological techniques to characterize the structure and function of certain sequences (e.g., the inverted terminal repeats) and genes of adenovirus (ad) DNA. In earlier studies, we first identified and characterized the VA RNA gene/transcript and inverted terminal repeats in Ad DNA. We have continued to investigate the specific functions of several early Ad genes, e.g., the VA and DNA-binding protein genes. Our results indicate that these latter genes are involved in the regulation of translation of certain viral mRNAs. Among methods used are gel electrophoresis, gene cloning, mutagenesis, base sequence analysis, and DNA transfection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00295-11 LVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Rose	Section Head	LVD, NIAID

COOPERATING UNITS *(if any)*
L. Mishra, Senior Staff Fellow, Division of Blood and Blood Products, CBER, FDA

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
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INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of this project are (i) to identify the helper virus genes that regulate expression of defective human parvovirus (AAV) genomes, and (ii) to define their respective roles in AAV replication. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. Among methods used are specific immunofluorescence, DNA cloning, gel electrophoresis, blot-hybridization analyses, and DNA transfection of both simian and human cell lines.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00296-11 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Pl:	J. A. Rose	Section Head	LVD, NIAID
Other:	S. Muralidhar	Visiting Associate	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

laboratory of Viral Diseases

SECTION

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INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.0	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of these studies are (1) to identify and characterize all proteins that are specified by the defective human parvovirus (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (2) to define the mechanism(s) by which the AAV proteins arise, and (3) to define specific functions of the AAV proteins. We have identified at least four AAV non-structural proteins. At least one of these proteins is necessary for viral DNA replication. Post-translational processing does not account for production of any AAV structural proteins although they share large segments of sequences-in-common. It is now clear, however, that these proteins originate from independent in-frame initiations. Mechanism that regulate expression of AAV proteins are of fundamental interest, and we have shown that one AAV structural protein is initiated by a codon (ACG) not known previously to act as an initiation codon in higher eukaryotes. Furthermore, our current findings (i) support a "scanning mechanism" in the translational expression of polycistronic eukaryotic mRNAs, and (ii) demonstrate that alternative mRNA splicing is required for effective translational expression of the largest AAV capsid protein. Among methods used are site-directed mutagenesis, affinity chromatography, gel electrophoresis, in vitro translation of viral RNA, DNA transfection, immunoprecipitation, and Western blotting.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00297-11 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism and Regulation of Adeno-associated Virus DNA Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P1:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Viral Diseases

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary objective of this project is to define molecular and biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) DNA replication in both in vivo and in vitro systems. We have shown that AAV DNA synthesis can be initiated in vitro in cell-free extracts and that replicating forms that correspond to those identified in vivo can be synthesized with either endogenous or exogenously added templates. Recently, we have shown that AAV "rep" proteins may not be required for the synthesis of AAV concatemeric DNA intermediates, but that one on more of these proteins is required to resolve the long intermediates into unit length duplexes. The observed mode of AAV DNA replication may serve as a model for telomere replication in eukaryotes. Among methods used are affinity chromatography, DNA sequence analysis, and restriction cleavage of DNA molecules and DNA transfection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER

Z01 AI 00298-12 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Development of Vaccinia Virus as an Expression Vector

PRINCIPAL INVESTIGATOR	B. Moss	Chief	LVD, NIAID
Others:	G. Sutter	Special Volunteer	LVD, NIAID
	L. Wyatt	Senior Staff Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

T. Fuerst, MedImmune

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.25	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The cytoplasmic site of gene expression and use of virally encoded enzymes is a distinguishing feature of vaccinia virus and other poxvirus vector systems that probably accounts for their consistent ability to express foreign genes derived from a variety of prokaryotic, eukaryotic, and viral sources. This feature, together with their ability to stably integrate and package large amounts of DNA without loss of infectivity, their wide host range, and the development of simple and effective methods for isolating recombinant viruses account for their diverse use and popularity.

During the past year, emphasis has been placed on the evaluation of the highly attenuated MVA strain of vaccinia virus as an expression vector. Because of the inability of MVA to replicate in human cells, it provides excellent safety. Following the demonstration of high levels of expression of a reporter gene in nonpermissive human cells, a series of transfer vectors was produced to allow vaccine testing in experimental animals. These tests indicate that recombinant MVA vectors provoke good immune responses and disease protection.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-604-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00306-12 LVD

PERIOD COVERED

October 1, 1990 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Orthopoxvirus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

	R. M. L. Buller	Expert	LVD, NIAID
Others:	G. Palumbo	Staff Fellow	LVD, NIAID
	G. Karupiah	Visiting Fellow	LVD, NIAID
	T. Koonin	Visiting Scientist	LVD, NIAID

COOPERATING UNITS (if any)

T. Fredrickson, NCI, NIH; D. Pickup, Duke University; T. Eling, NIEHS; Kerry Oliver, NIEHS; R. Drillien, U. of Louis Pasteur, France; E. Brownell, Miles Research, Inc.; M. Spriggs, Immunex Research and Development Corp; D. Hruby, Oregon State University

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project, we focused our studies on the genetic basis both of poxvirus virulence and of host resistance to virus infection. The acquired knowledge should contribute toward development of safe, effective recombinant vaccinia virus vaccines for animal and human use.

The genetic basis for ectromelia virus' narrow host range is poorly understood. Analyses of the tissue tropism genes CHO, K1L, and C7L revealed that the CHO gene is inactive and the K1L and C7L genes have only partial activity. Deletion of the K1L gene had no detectable effect on ectromelia virus pathogenesis in the mouse. These observations are consistent with the narrow host range of ectromelia.

A newly identified EV ORF, predicted to encode a 28 kDa protein, was shown to interact with DNA—presumably through a zinc finger motif in the carboxyl terminal region of the protein. The protein was expressed at low levels in the infected cell, and preliminary evidence suggested a nuclear site of localization. A 28 kDa$^-$ mutant replicated normally in tested tissue culture cell lines, but at least 100-fold less efficiently in tissues from the infected mouse and spread more slowly from the primary site of infection to spleen and liver. The LD_{50} of the mutant virus was at least 1000-fold lower than the WT. This gene appears to be an important poxvirus virulence determinant in the mousepox model.

Treatment with IFN-γ allowed mouse macrophages to restrict the replication of ectromelia, vaccinia, and herpes simplex viruses while producing nitric oxide. Inhibitors of nitric oxide synthase blocked the macrophages' antiviral activity. Virus replication was diminished in epithelial cells transfected with a cDNA encoding inducible nitric oxide synthase or treated with organic compounds that generate nitric oxide. In mice, an inhibitor of nitric oxide synthase converted sublethal, resolving ectromelia virus infection into fulminant mousepox. Thus, induction of nitric oxide synthase was necessary and sufficient for a substantial antiviral effect of IFN-γ *in vitro* and *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER

Z01 AI 00307-12 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Regulation of Vaccinia Virus Gene Expression

PRINCIPAL INVESTIGATOR	B. Moss	Laboratory Chief	LVD, NIAID
Others:	B.-Y. Ahn	Visiting Associate	LVD, NIAID
	C. J. Baldick	IRTA	LVD, NIAID
	P. Gershon	Visiting Associate	LVD, NIAID
	J. Keck	Staff Fellow	LVD, NIAID
	N. Harris	Visiting Fellow	LVD, NIAID
	R. Rosales	Visiting Fellow	LVD, NIAID
	B. Schnierle	Volunteer	LVD, NIAID
	L. Carroll	IRTA	LVD, NIAID
	G. Kovacs	IRTA	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	7.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Poxviruses, unlike other DNA viruses, replicate in the cytoplasm of the cell and encode most or all of the enzymes and factors needed for transcription of their genomes. Vaccinia virus provides a unique system for combining biochemical and genetic approaches for investigating mechanisms of gene regulation, and has led to many basic discoveries.

Studies with vaccinia virus indicated that the genes are divided into three temporal classes—early, intermediate, and late—that are regulated in a cascade fashion. By infecting cells in a highly synchronous manner, the waves of early, intermediate, and late mRNA were clearly demarcated for the first time. Emphasis during the past year was placed on the factors required for intermediate and late transcription. The role for the capping enzyme in initiation of intermediate transcription was shown to be independent of its RNA guanylytransferase function. *In vivo* studies supported the role of the product of the G8R gene in late transcription and further showed that it is not required for intermediate transcription. The 17 kD product of the A1L gene was shown to be a transcription factor and to bind zinc. Another late transactivator protein of 26 kD encoded by the A2L gene also was shown to be a zinc-binding protein. Moreover, mutations of the zinc-binding domain abrogated the transcriptional transactivation property of the factor.

The mRNAs of vaccinia virus, like those of eukaryotes, contain a 3′ poly(A) tail. Further studies of the vaccinia virus-encoded poly(A) polymerase demonstrated that the small VP39 subunit enhances activity by converting the slow non-processive reaction to a rapid semi-processive one.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH**	PROJECT NUMBER Z01 AI 00416-10 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Recombinant Vaccines against Retrovirus Associated with Leukemia and AIDS

PRINCIPAL INVESTIGATOR	B. Moss	Laboratory Chief	LVD, NIAID
Others:	P. Earl	Microbiologist	LVD, NIAID
	V. Karacostas	Pre-IRTA	LVD, NIAID
	C. Broder	IRTA	LVD, NIAID

COOPERATING UNITS *(If any)*

R. Doms, University of Pennsylvania

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS). At present, there is no effective vaccine against this disease, and therapeutic agents provide only limited help. The objects of this project are to characterize HIV antigens, to determine the targets of humoral and cell-mediated immunity, and to use this information to develop candidate vaccines. We have constructed recombinant vaccinia viruses containing HIV genetic information. These viruses have been used as live experimental vaccines to immunize animals, to synthesize HIV proteins in tissue culture, to make targets for cytotoxic T cells, and to study CD4-envelope protein interactions.

Our previous studies indicated that the native form of the HIV-1 envelope protein is a tetramer composed of two dimers. Mutagenesis studies demonstrated that amino acids 68 to 129 of gp41 are essential for oligomerization. The binding region appears to be quite broad, since point mutations within highly conserved amino acids were without effect. Monoclonal antibodies were produced that reacted preferentially or exclusively with oligomeric forms of the envelope protein.

Expression of the HIV-1 *gag-pol* gene by a recombinant vaccinia virus was previously shown to result in the budding of retrovirus-like particles that contained active reverse transcriptase. Increasing the amount of the gag-pol protein caused premature activation of the protease and inhibition of particle formation. This assembly block could be reversed by treating the cells with a protease inhibitor or by mutagenizing the protease active domain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI-00445-09
LVD

PERIOD COVERED October 1, 19911 to September 30,1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Viral DNA Replication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. D. Challberg	Section Head	LVD, NIAID
Others:	D. Fierer	Medical Fellow	LVD, NIAID
	J. Gottlieb	IRTA Fellow	LVD, NIAID
	G. Sherman	IRTA Fellow	LVD, NIAID
	D. Klinedinst	IRTA Fellow	LVD, NIAID
	W. Zhen	Visiting Associate	LVD, NIAID

COOPERATING UNITS (if any)

Department of Molecular Biology and Genetics, Johns Hopkins Medical School
Institute of Virology, University of Glasgow

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION N

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.2	5.5	1.7

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews
_(b) Human tissues
x _(c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are engaged in analysis of the lytic replication of two human herpesviruses: herpes simplex virus (HSV) and Epstein-Barr virus (EBV). We are studying the purified protein products of the viral genes that participate in DNA replication in lytically-infected cells, using both biochemical and molecular genetic approaches to understand the function of these polypeptides in detail.

Our recent results regarding HSV proteins can be summarized as follows. 1)UL9, the viral protein that presumably initiates DNA replication, interacts specifically with ICP8, the virus enxoded single-stranded DNA binding protein. 2) The products of the HSV genes UL5, UL8, and UL52 form a three polypeptide complex that has both helicase and primase activities. We have mapped the regions of UL52 that are essential for interaction with UL5 and UL8, and have obtained preliminary evidence that UL52 is essential for primase catalysis. 3) The HSV DNA polymerase consists of a stable complex of two polypeptides: UL30, the catalytic subunit, and UL42, an accessory subunit that increases the processivity of the enzyme. Our results have shown that the increase in processivity brought about by UL42 does not result in an increase in the fidelity of DNA synthesis. 4) We have reconstituted rolling circle DNA replication in vitro using purified HSV replication proteins. Since this reaction depends on 6 of the seven viral gene products required in vivo, we are exploiting this system to gain insight into how these polypeptides interact at a replication fork. In addition, we have developed an assay to investigate the involvement of cellular proteins in viral DNA replication

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00538-6 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Interaction of Human Immunodeficiency Virus with the CD4 Receptor

PRINCIPAL INVESTIGATOR	E. Berger	Microbiologist	LVD, NIAID
Others:	B. Moss	Laboratory Chief	LVD, NIAID
	C. Broder	NRC Associate	LVD, NIAID
	O. Nussbaum	Visiting Associate	LVD, NIAID
	P. Kennedy	Microbiologist	LVD, NIAID

COOPERATING UNITS *(if any)*

NCI (I. Pastan, D. FitzGerald, F. Michaels); NIAID (P. Bressler); NHLBI (R. Morgan).

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	**PROFESSIONAL:**	**OTHER:**

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

CD4 is a surface molecule that serves as the receptor for human immunodeficiency virus (HIV), the causative agent of AIDS. We are studying two areas related to CD4 and HIV: *1) Mechanisms of membrane fusion mediated by the CD4/HIV* env *interaction*. One focus of this work is to identify accessory components of CD4$^+$ target cells which determine the specificity of HIV fusion. Two types of specificities are under study: the selective tropism of different HIV-1 isolates for T cell lines *vs.* primary macrophages, and the ability of human CD4 to mediate fusion when expressed on a human cell but not on an animal cell. To study these problems, we have developed a novel vaccinia-based assay in which fusion between CD4$^+$ and *env*$^+$ cells leads to lacZ gene activation by phage T7 RNA polymerase and consequent accumulation of β-galactosidase in the cytoplasm of the fused cells. The rapidity, sensitivity, and flexibility of this assay make it highly suitable for mechanistic studies and for screening fusion-blocking antibodies and drugs. Using this assay, we have obtained the first direct evidence that the replication tropism of different HIV-1 isolates for T cell lines *vs.* primary macrophages is determined primarily by the specificity of *env*-mediated fusion with the target cell. Regarding the species specificity, we have demonstrated functional transfer the human cell-specific accessory component(s) to animal cells using PEG-mediated membrane vesicle fusion. The identities of the cellular components associated with specificity of *env*/CD4-mediated fusion are a major focus for present work. We also observed that co-expression of the protease furin greatly stimulates *env*/CD4 fusion in the vaccinia system, presumably by enhancing processing of the gp160 precursor. This finding greatly enhances the cell fusion assay system and should help circumvent problems which have compromised a second focus of this work, namely analysis of structural features of *env* and CD4 in fusion. By co-expressing furin, we expect to be able to produce large quantities of correctly processed *env* glycoprotein (membrane-associated and soluble forms) for structure/function studies. *2) CD4-based therapeutics for treatment of HIV infection*. We have designed a genetically engineered hybrid toxin (CD4-PE40) which selectively kills HIV-infected cells and is active against both laboratory-adapted and primary HIV-1 isolates. We are currently testing whether the dose-limiting hepatotoxicity observed in initial Phase I clinical trials is associated with gp120 binding to CD4-PE40 and consequent enhanced uptake into liver cells. Studies are also in progress to test the ability of CD4-PE40 (alone or in combination with other antiretroviral agents) to eliminate HIV *ex vivo* from infected PBLs and bone marrow cells obtained from HIV-positive individuals. Such methods may prove useful in genetic therapy and bone marrow transplantation approaches to combat AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH**	PROJECT NUMBER Z01 AI 00539-06 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT

Virus-Cell Interactions, Viral Pathogenesis, and Host Immunity

PRINCIPAL INVESTIGATOR	B. Moss	Laboratory Chief	LVD, NIAID
Others:	B-Y. Ahn	Visiting Associate	LVD, NIAID
	A. Ramsey-Ewing	IRTA	LVD, NIAID
	R. Roper	IRTA	LVD, NIAID
	E. Wolffe	IRTA	LVD, NIAID

COOPERATING UNITS *(if any)*

R. W. Doms, University of Pennsylvania; G. Griffiths, European Mol. Biol Lab; R. Jagus, University of Maryland

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

A successful virus infection involves entry into the cell, uncoating, expression and replication of the genome, assembly and release of infectious virus particles, and defense against specific and non-specific host immune mechanisms. Combined genetic, biochemical, electron microscopic, and immunological approaches are being used to investigate these complex processes.

During the past year, collaborative studies led to a new model of vaccinia virus assembly in which membranes of the immediate cellular compartment enwrap viral proteins and DNA. In addition, two glycoprotein components of a second wrapping membrane, which is reuqired for virus release from the cell, were characterized. The 42 kD product of the B5R gene was shown to be a class I membrane glycoprotein component of the extracellular form of vaccinia virus. Deletion of the B5R gene resulted in a highly attenuated mutant virus that was severely impaired in envelope formation and cell-to-cell dissemination. The 21 kD product of the A34R gene was found to regulate the release of extracellular virions from the plasma membrane. A virion-associated protein with homology to glutaredoxin was found to have thioltransferase activity, suggesting that it may have novel roles in the virus growth cycle.

The vaccinia virus-encoded complement control protein (VCP) serves as a defense against the host immune system. Studies on the action of VCP indicated that it binds C4b and C3b and interferes at several steps in the classical and alternative pathways of complement activation. An interferon defense molecule, the double-stranded DNA binding protein encoded by the E3L gene, was shown to localize within the nuclei of cells, suggesting an additional role.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00540-05-LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Human Herpesviruses 6 and 7

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Frenkel	Head Section	LVD, NIAID
Others:	L. Wyatt	Senior Staff Fellow	LVD, NIAID
	B. Avidor	Visiting Associate	LVD, NIAID
	E. Roffman	Visiting Associate	LVD, NIAID
	B. Lum	IRTA Fellow	LVD, NIAID
	R. Danovich	NRC Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

Dr. D. Di Luca, University of Ferrara, Italy.
Dr. J. Sever, Children's Hospital, Washington, DC.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	2.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This report concerns the structure and function of human herpesviruses 6 and 7, their interactions with HIV, and interactions with T cells. We have recently shown that virus strains previously recognized as HHV-6, isolated in the USA, Europe, and Japan, fall into two distinct categories. These virus groups have been tentatively designated as Exanthem Subitum Virus (or HHV-6) and HHV-8. HHV-7 was isolated in our laboratory in 1989, from CD4+ T cells of a healthy individual. Our studies this past year concerned several topics: (i) we have continued the derivation of approaches to be used in studies of HHV-7. Further cloning and mapping of HHV-7 DNA has been pursued. Sequences of the junction between the direct terminal repeat and the unique sequences have been analyzed. PCR primers have been derived and tested for specificity. (ii) We have continued our studies relevant to virus-host cell interactions. Our previous studies included analyses of the effect of T cell activation on virus replication, activation of HHV-6 and HHV-7 from latency, the effect of virus infection on T cell function, and analyses of parameters which determine entry into latency. We have analyzed HHV-6 sensitivity to interferon. We have shown the conditions for activation of HHV-6 and HHV-7 from latency. Entry and reactivation from latency are of potential medical importance, in as much as viral reactivation occurs in organ transplant and bone marrow transplant patients undergoing immunosuppression. (iii) In a study currently in progress, in collaboration with Dr. D. DiLuca of University of Ferrara, we have investigated the prevalence of HHV-7 DNA in Hodgkin's and non-Hodgkin's lymphomas. (iv) In recent studies, in collaboration with Dr. Sever of Childrens' Hospital, we have investigated whether HHV-6 plays a role as a cofactor, in the rapid development of AIDS in children to HIV+ mothers. Preliminary results have been obtained and future prospective studies have been designed. (v) We are attempting to characterize the microenvironment of the CD4 molecule on T cells. Further studies are planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00541-06 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Folding, Assembly, and Transport of Viral Glycoprotiens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID

COOPERATING UNITS *(if any)*

T. Bachi, Electron Microscopy Laboratory, Zurich, Switzerland
H-P. Hauri, Biozenter, Basle, Switzerland

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.40 staff years	0.20 staff years	0.20 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The improvement of current anti-viral vaccines and the development of novel vaccines depends on increasing our understanding of viral attachment and fusion glycoproteins. Critical insight into understanding the antigenic structure of glycoproteins is provided by studying their interaction with monoclonal antibodies (mAbs). For a number of years we have studied the influenza virus hemagglutinin (HA) glycoprotein. This protein serves as a model for other proteins with similar functions (e.g. HIV gp160), and moreover, is important practically in its own right, as influenza still is a major cause of morbidity and mortality nationally, and internationally.

Like many viral glycoproteins the HA is a homo-oligomer, consisting of three identical monomeric subunits. In the past year we continued to investigate the site of trimerization of newly synthesized HA. Our previously published findings suggested that HA trimerization occurs only after monomers are exported from the ER. This conclusion was based largely on localization studies performed with a standard immunofluorescence microscope. In the past year we have increased the resolution of detection by using a laser confocal scanning microscope to detect fluor-tagged antibodies, and an electron microscope to detect colloidal gold tagged antibodies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00542-06

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Processing of Viral Proteins for T cell Recognition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.W. Yewdell		Medical Officer (Research)	LVD, NIAID
J.R. Bennink		Microbiologist	LVD, NIAID
Others:	Patricia Day	IRTA Fellow	LVD, NIAID
	Cheryl Lapham	IRTA Fellow	LVD, NIAID
	Yuping Deng	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

Jan Lukso, BRB, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.90 staff years	0.20 staff years	2.70 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Class I molecules of the major histocompatibility complex (MHC) consist of a highly polymorphic heavy chain complexed to b microglobulin (bm). Class I molecules are expressed on virtually all cell types. Their sole function is to bind antigens and present them to T cell bearing CD8 molecules (T_{CD8+}). T_{CD8+} play a critical role in eradicating intracellular pathogens and tumors. They are also a significant cause of immunopathology, being involved in organ rejection and autoimmune diseases.

There has been rapid progress in understanding the physical nature of the antigen-class I complex and in how antigens are generated and become associated with class I molecules in cells. It is now apparent that antigens derived from a cytosolic pool of proteins are translocated into an exocytic compartment by a MHC encoded transporter complex. Once transported from the cytosol, antigens bind to class I molecules and are transported to the cell surface.

In the past year we have continued our studies on the assembly and trafficking of MHC class I molecules and have made progress on a number of fronts:

1) we obtained the first direct evidence that peptides associate with class I molecules in an early exocytic compartment,

2) we developed fluorescent derivatives of an antigenic peptides and used these probes to make several novel discoveries about the intracellular trafficking of class I molecules and antigenic peptides,

3) we developed a novel quantitative assay for class I molecule expression and used this assay to gain new insights into class I trafficking in cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00564-04 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Parvovirus Vectors that Regulate Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P1:	J. A. Rose	Section Head	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was designed to develop adeno-associated virus (AAV)-based vectors that could down-regulate expression of specific viral genes by mediating the production of appropriate antisense RNA molecules. Among targets chosen for study are certain early viral gene products which are absolutely necessary for productive viral infection. A series of AAV-based vectors has been created in which the endogenous AAV promoters and coding sequences were replaced with a polylinker and several strong constitutive and inducible heterologous promoters. An encapsidation system has been developed in orders to produce high titres of infectious recombinant virus. The induction of resistance to both HIV-1 infection by such vectors is currently being studies, and present results indicate that a 95% reduction in the replication of either viruses can be achieved. Methods used include: gene cloning, transfection of cells, radio-innunoprecipitation, immunofluorescence, RNA and DNA hybridization techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00619-03 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intracellular Antibody-mediated Virus Neutralization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.R. Bennink	Microbiologist	LVD, NIAID
J.W. Yewdell	Medical Officer (Research)	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.00 staff years	0.00 staff years	0.00 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the greatest hurdles in creating vaccines for a number of viral pathogens is the antigenic variability of viral coat proteins. The rapid mutation rate of a number of viruses (most notably influenza virus and HIV) allows the virus to escape the neutralizing antibody response induced by anti-viral vaccines. The internal proteins of the virus, on the other hand, is generally highly conserved, and have not evolved to rapidly alter their antigenicity. Antibody responses to these internal proteins, while a consistent feature of immune responses to viruses, almost always fail to influence viral infectivity, since the antibodies do not access to their target antigens, which are located either inside the virus, or inside the virus infected cells. If, however, antibodies are introduced into the cytosol of cells, they do have the ability to prevent viral infection. Recent advances in understanding antibody folding indicate that antibodies can under some circumstances properly fold and bind antigen when they are expressed in the cytosol by removing their amino terminal ER insertion sequences. Therefore, cells, or eventually, transgenic animals expressing such cytosolic antibodies should be resistant to virus infection. Using electroporation to introduce antibodies into the cytosol, we identified antibodies able to block infection of cells with influenza A or B viruses. Our goal is to clone the genes encoding these antibodies and express them in the cytosol of tissue culture cells, and eventually transgenic animals, thus creating animals resistant to influenza virus infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00620-02-LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Epidemiology of Human Herpesviruses 6 and 7

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Frenkel	Section Head	LVD, NIAID
Others:	L. Wyatt	Senior Staff Fellow	LVD, NIAID
	G. Katsafanas	Biologist	LVD, NIAID
	T. Butler	Biologist	LVD, NIAID

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Y. Asano. Fugita Health University, School of Medicine, JAPAN

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Molecular Genetics Section

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project concerns the new lymphotropic human herpesviruses 6 (HHV-6), HHV-7 and HHV-8. The overall goal of these studies has been to determine virus involvement in human disease(s). The results of these studies during the past year can be summarized at follows: (i) HHV-6 and HHV-8. Characterization of multiple virus isolates have revealed that the herpesviruses previously designated as HHV-6 fall into two distinct categories. These viruses differed in restriction enzyme pattern of viral DNA, antigenically and epidemiologically. As per these studies, and confirmed by others, these viruses will be classified as Exanthem Subitum Virus (ESV), prototyped by the HHV-6 Z29 strain, and HHV-8, prototyped by the U1102, or by the GS strains. (ii) Disease association of ESV or HHV-6. In addition to viruses from ES patients, we have also analyzed viruses from patients with fatal encephalitis and fatal fulminant hepatitis. All of these viruses were similar to ESV strain Z29. They were distinct from the U1102 strain, as well as from HHV-7 DNA. (iii) Virus reactivation from latency: Analyses of viruses from 8 renal transplant patients and 10 bone marrow transplant patients revealed similarity to the Z29 virus, except minor strain variations in the terminal repeat sequences. In one case of renal transplant the reactivated virus originated from the donor kidneys, in as much as identical viruses were derived from two transplanted kidneys from the same donor. In ES patients, virus can only be recovered during 3-4 days of the acute (high fever) phase, in BMT patients virus could be repeatedly isolated for lengthy time, revealing long term viremia. We conclude that ESV (rather than HHV-8), can be associated with morbidity and mortality in immunocompromised patients. Further studies are designed with additional clinical centers, in the USA, Japan and Israel, to extend these conclusions, as well as to allow early detection of reactivated virus, for more timely therapy with potent anti-viral drugs which are too toxic for lengthy therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00652-02 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Peptide Transporters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID
Others: Gustav Russ	Visiting Associate	LVD, NIAID

COOPERATING UNITS *(if any)*

T. Spies, Dana Farber Cancer Institute, Boston, MA
R. Demars, University of Wisconsin, Madison, WI
P. Cresswell, Yale University, New Haven, CT

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.40 staff years	0.20 staff years	1.20 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD8+ T cells (T_{CD8+}) play an important role in controlling virus infections. T_{CD8+} recognize peptides of 8 to 10 residues derived from viral proteins located in the cytosol of virus infected cells. These peptides are recognized in a complex with class I molecules encoded by the major histocompatibility complex (MHC). In the past 3 years it was discovered that the MHC also encodes two molecules, termed TAP1 and TAP2, that seem to specifically transport peptides from the cytosol into the intracellular compartment that contains class I molecules. TAP genes have been found to be polymorphic in humans, although to a far lesser extent than the class I molecules. The existence of these putative peptide pumps and their polymorphism raises a number of important questions: Where in the cells are the pumps located? Do the pumps influence the types of peptides presented by class I molecules? Are the pumps tethered to the proteases that produce antigenic peptides in the cytosol? How do the pumps work? Are there individuals with immune deficiencies based on mutations in the TAP genes? Can cells transport peptides via other mechanisms?
To help characterize the structure and function of the TAP genes we have inserted them into vaccinia virus. We have demonstrated that a Vac recombinant encoding TAP1 produces a fully functional gene, and have found that TAP1 produced by this recombinant localizes to the endoplasmic reticulum where it is glycosylated. We produced a recombinant that produces a functionally active TAP2. We have also examined the specificity of the antigen transporters. We found that the transporters can transport the exact peptides presented by class I molecules. Also we examined whether expression of the human TAP2 gene in mouse cells adversely affects the ability of the cells to present viral proteins to T_{CD8+}. We find that the human TAP2 gene functions in a indistinguishable manner from its mouse counterpart. Finally, we have explored the presentation of peptides in cells that fail to express one or both TAP genes.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00653-02 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Delivery of Antigens to the MHC Class I Processing Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

	J.R. Bennink	Microbiologist	LVD, NIAID
	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
Others:	R. Anderson	NRC Fellow	LVD, NIAID
	I. Bacik	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

B. Murphy, LID, NIAID, NIH, N. Restifo, Surgery Branch, NCI, NIH
C. Leamon and P. Low, Purdue University, W. Lafayette, IN
V. Nussenweig, New York University, New York, NY

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.90 staff years	0.20 staff years	1.70 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD8 positive lymphocytes (T_{CD8+}) play an important role in host immunity to viruses and other intracellular parasites. Anti-viral T_{CD8+} recognize peptides derived from a cytosolic pool of viral proteins. Induction of anti-viral T_{CD8+} responses is limited by the requirement that the antigen be delivered to the cytosol of antigen presenting cells *in vivo*. Using infectious vaccines, this is accomplished as part of the infectious cycle of the immunogen. Non-infectious vaccines (inactivated virus, subviral preparations) often fail to induce T_{CD8+} responses due to their inability to deliver antigens to the cytosol. It should, however, be possible to induce T_{CD8+} responses to non-infectious antigens if the antigen is modified in such a way as to allow its efficient delivery to the cytosol. To explore this vaccine strategy we studied a model antigen (ovalbumin) that we chemically conjugated to folic acid. By targeting proteins to folic acid receptors, it is believed that this modification facilitates the delivery of proteins to the cytosol. In the past year we studied the presentation of folic acid-conjugated ovalbumin to ovalbumin-specific T_{CD8+}. We failed to obtain evidence that folic acid is able to deliver ovalbumin to the class I antigen processing pathway. In an effort to develop other novel vaccines for eliciting T_{CD8+} we created a number of recombinant vaccinia viruses that express antigenic peptides in the absence of flanking residues or at the COOH terminus of a sequence that directs the peptides to the endoplasmic reticulum. Preliminary testing of these recombinants in two distinct systems indicates that these recombinants more efficiently elicit T_{CD8+} responses than traditional recombinants expressing full length gene products.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00658-02 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Proteolytic generation of antigenic peptide

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.R. Bennink		Microbiologist	LVD, NIAID
J.W. Yewdell		Medical Officer (Research)	LVD, NIAID
Others:	Josephine Cox	Visiting Scientist	LVD, NIAID
	Igor Bacik	Visiting Fellow	LVD, NIAID
	Luis Anton	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

T. Spies, Dana Farber Cancer Institute, Boston, MA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.40 staff years	0.20 staff years	3.20 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD8 expressing T cells (T_{CD8+}) play a critical role in eradicating intracellular parasites such as viruses. T_{CD8+} recognize MHC class I molecules in a complex with peptides of 8 to 10 residues derived from viral proteins located in the cytosol. There is tremendous interest in the mechanism by which peptides are generated. There is indirect evidence that implicates proteasomes in the generation of antigenic peptides. Proteasomes are abundant macromolecular structures present in the cytosol and nucleus in cells, and have multiple protease activities. They are thought to be responsible for energy dependent proteolysis in which ubiquitin plays a prominent role in targeting proteins for destruction. Although it is believed that at least some proteolysis occurs in the cytosol, it is uncertain whether the ultimate determinants are generated in the cytosol or whether additional trimming occurs following transport from the cytosol.

We have initiated several approaches to assess the site and nature of proteolytic mechanisms utilized in the generation of antigenic peptides. First, we demonstrated that two MHC encoded proteins that are present in the proteosomes are not required for efficient antigen presentation. Second, we produced vaccinia recombinants expressing a form of ubiquitin altered to interfere with ubiquitin-targeted proteolysis. Third, we have investigated the antigen presentation capacity of mutant cells deficient in ubiquiting conjugation. Fourth, we initiated a project designed to determine whether cellular proteins are selectively spared from providing antigenic peptides by the antigen processing machinery.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00659-02 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen presentation by human tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.R. Bennink	Microbiologist	LVD, NIAID
J.W. Yewdell	Medical Officer (Research)	LVD, NIAID

COOPERATING UNITS *(if any)*

N. Restifo, Surgery Branch, NCI
S. Rosenberg, Surgery Branch, NCI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.40 staff years	0.20 staff years	0.20 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is a considerable body of evidence that tumor specific CD8+ T lymphocytes (T_{CD8+}) can be elicited in tumor bearing individuals and can mediate a significant anti-tumor effect. In many, if not most cases, however, tumor specific T_{CD8+} do not arise naturally. We are investigating the possibility that the poor immunogenicity of some tumors is due to limitations in the abilities of cancer cells to present their tumor-specific antigens to the immune system.

In the past few years there has been tremendous progress in our understanding of how cells present antigens for recognition by T_{CD8+}. It is now clear that presentation of antigen results from a chain of events, including protein breakdown into peptides of 8 to 10 amino acids, transport of peptides from the cytosol into the endoplasmic reticulum, binding of peptides to a special peptide-presenting complex, and delivery of the presenting molecule to the cell surface. To test the hypothesis that limitations in tumor antigen breakdown or transport of the antigen into the endoplasmic reticulum limit the stimulation of T cells, we have created novel vaccines in which antigenic peptide generation and transport occurs independent of the normal antigen processing machinery. We are currently testing whether such vaccines are able to elicit tumor specific T_{CD8+} and protect animals against a challenge with tumor cells.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00660-02 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Brefeldin A action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID
Others: Yuping Deng	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

R. Haugland, Molecular Probes, Eugene, OR
H.C. Kang, Molecular Probes, Eugene, OR

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.90 staff years	0.20 staff years	0.70 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Brefeldin A (BFA) is a fungal metabolite with a broad range of biological activities, including the anti-fungal and anti-tumor activities. In the past few years there has been tremendous interest in its effect on cells, since it has been found to have the unique ability to interfere with vesicular trafficking. Among its effects, it has the ability to disperse the Golgi complex, returning at least some of its components to the endoplasmic reticulum (ER). BFA also prevents newly synthesized proteins destined for the cell surface from leaving the ER. To better understand the mechanism of BFA action we conjugated it to two fluorescent dyes and studied its intracellular localization. Both conjugates maintained their biological activity, and both specifically localized to the ER and Golgi complex in live or aldehyde-fixed cells. The interaction with intracellular membranes is probably due to interaction with lipids as it is abolished by detergent extraction of lipids. These conjugates are the first dyes that bind the ER and Golgi complex without binding other prominent membrane bound compartments and should prove useful as probes for the these organelles in living cells. Their ability to stain the ER of fixed cells will be of great use as markers of the ER in cytochemical studies. Finally, the interaction of BFA with the ER and GC membranes might be critical to its biological activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00661-02 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Confocal Microscopy of Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID

COOPERATING UNITS *(if any)*

K. Kelly, LP, DCBD, NCI
L. Staudt, MET, NCI
R. Germain, LI, NIAID
P. Matzinger, LCMI, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.40 staff years	0.20 staff years	0.20 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent advances in optics and electronics have culminated in the development of the laser confocal scanning microscope (LCSM). The LCSM provides unparalleled resolution for localizing fluorescent probes in cells. In combination with advanced, yet relatively inexpensive and compact computers, it is relatively easy to produce 3-dimensional reconstructions of fluorescently labelled structures in cells. We have been working with a state of the art BioRad MRC 600 LCSM with its controlling computer. To produce 3-D reconstructions of data sets obtained with the LCSM we use VoxelView software on a Silicon Graphics Iris Indigo computer. We have used this system for a wide variety of applications, including determining the subcellular distribution of newly defined proteins, following the intracellular trafficking of newly synthesized membrane proteins, studying the migration of developing thymocytes in thymic explants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00690-01 LVD

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigen Processing in Lower Eukaryotic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID
Others: Yuping Deng	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Cellular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.90 staff years	0.20 staff years	0.70 staff years

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD8+ T cells (T_{CD8+}) recognize class I molecules of the major histocompatibility complex (MHC) bearing peptides of 8 to 10 residues derived from cytosolic proteins. These cells are a bulwark of host defenses to infectious agents and tumors and if we are to improve existing vaccines and develop new vaccines and treatments for infectious and neoplastic diseases, it is critical to understand *antigen processing*, the mechanism by which antigenic peptides are generated by cells and delivered to class I molecules. Although there has been great progress in understanding antigen processing in the past 5 years, there remains much to be learned. The strategy of this project is to delineate what we *don't* know. We are expressing the known constituents of the antigen processing machinery in mosquito cells, and determining whether this is sufficient to reconstitute antigen processing to a level seen in mammalian cells. Insects do not have a MHC, and do not therefore, have any of the specialized machinery associated with antigen processing. If these cells can process antigens under these conditions, this would indicate that we have identified the major components of antigen processing, if they can't, it would mean that further discoveries are required. In the past year we made considerable progress in setting up a workable system, and are poised to gain solid information regarding this question.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-00691-01 LVD

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Proteolytic Processing of Antigenic Peptides in the Endoplasmic Reticulum

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.R. Bennink		Microbiologist	LVD, NIAID
J.W. Yewdell		Medical Officer (Research)	LVD, NIAID
Others:	Heidi Link	IRTA Fellow	LVD, NIAID
	Igor Bacik	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.90 staff years	0.20 staff years	1.70 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD8+ T cells (T_{CD8+}) recognize class I molecules of the major histocompatibility complex (MHC) bearing peptides of 8 to 10 residues derived from cytosolic proteins. These cells are a bulwark of host defenses to infectious agents and tumors, and it is critical to understand how antigenic peptides are generated by cells if we are to improve existing vaccines and develop new vaccines and treatments for infectious and neoplastic diseases. Very little is known about how cells produce antigenic peptides from proteins. Previous results from our laboratory indicate that the endoplasmic reticulum (ER) is capable of trimming longer peptides that are imported from the cytosol. We have used a novel strategy to characterize the proteolytic capacity of the ER and its relevance to antigen processing. Our findings suggest that the ER is capable of trimming peptides to the sizes preferred by MHC class I molecules.

LABORATORY OF VECTORS AND PATHOGENS
Rocky Mountain Laboratories
Hamilton, Montana
1993 Annual Report
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ANNUAL REPORT
LABORATORY OF VECTORS AND PATHOGENS
HAMILTON, MONTANA
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1992, TO SEPTEMBER 30, 1993

Members of the Laboratory of Vectors and Pathogens have continued their molecular dissection of important human pathogens during the past year. This multidisciplinary approach to microbial pathogenesis combines the fields of biochemistry, immunology, electron microscopy, medical entomology and molecular biology into a cohesive unit with strong interaction among its own members and with scientific units both inside and outside of the NIH system. This interaction together with an active seminar and guest researcher program insures that the best experimental approaches available are rapidly incorporated into the research programs of the laboratory. The Laboratory of Vectors and Pathogens functions in four experimental groups emphasizing several pathogenic genera: *Borrelia*, *Campylobacter*, *Escherichia*, *Yersinia*, *Shigella*, and *Salmonella*. Recently, another pathogen of major human health importance has been added - *Mycobacterium tuberculosis*. Dr. Claude F. Garon serves as Laboratory Chief.

The Arthropod-borne Diseases Section, under the direction of Dr. Tom G. Schwan, has focused on the use of both molecular and classical techniques to investigate pathogen-arthropod interactions. *Yersinia pestis*, the bacterial agent of plague, primarily infects small mammals and is cycled from infected to uninfected hosts by fleas. The ecology of plague is complex. Over 200 different species of mammals and at least 80 different species of fleas have been implicated in maintaining *Y. pestis* in enzootic foci throughout the world. Plague is well established in certain wild rodent populations in parts of the western United States. Humans can acquire plague through contact with infected mammals or fleas. In the United States, most cases of plague in humans occur during periodic epizootics of plague among wild rodents. For this reason, determination of the prevalence and distribution of *Y. pestis* in these animal populations and their fleas is an important part of plague surveillance. Fleas are often preferred specimens because they are easier and safer to handle than mammalian tissue. The standard public health laboratory methods for plague surveillance have changed little in the 100 years since they were pioneered by Yersin and Kitasato: inoculation of experimental animals with suspect flea or animal tissue and subsequent isolation and identification of *Y. pestis*. These methods are reliable but require weeks for their completion and the use of laboratory animals. Recently, a DNA probe hybridization method was developed by members of the Arthropod-borne Diseases Section to detect *Y. pestis* in fleas. However, the DNA probe method only detects fleas infected with 10^5 or more bacteria. A more rapid and sensitive alternative method for the direct detection of *Y. pestis* cells in infected fleas using the polymerase chain reaction (PCR) was developed. The PCR test uses primers designed from the *Y. pestis* plasminogen activator gene to directly detect plague-infected fleas. As few as 10 *Y. pestis* cells were detected, even in the presence of flea tissue, by PCR and then agarose gel electrophoresis and ethidium bromide staining. The feasibility of the assay was demonstrated by using naturally infected *Xenopsylla cheopis* fleas. The detection of *Y. pestis* in fleas by PCR provides a rapid and sensitive way to monitor plague in wild animal populations, allowing public health officials to better assess the potential risk of transmission to humans. The PCR method has advantages over other available methods for the detection of *Y. pestis* in fleas. It is simple to perform, produces results within eight hours, and detects small numbers of bacteria without the need for laboratory animals or DNA probe labelling techniques. This method should be useful in plague surveillance studies and could be applied to other types of specimens. It may also prove to be a valuable tool in examining the interaction between *Y. pestis* and its insect host. (Schwan, Hinnebusch)

The Structural Pathobiology Section, under the direction of Dr. Claude F. Garon continues its molecular dissection of the Lyme disease spirochete. Lymphocyte blastogenesis assays and immunoblotting were used to investigate and compare murine B-cell responses to preparations of extracellular membrane blebs (BAg) and spirochetes (Ag) of *Borrelia burgdorferi*. Immunoblotting BAg, Ag and medium control preparations with serum from naive and infected C57BL/10 mice revealed similar specific reactivity profiles between BAg and Ag, but in contrast to Ag, major antigens of 83, 60 and 41 kDa were not detected in BAg. It was determined that 1 μg dry weight of Ag contained 0.0051 and 0.0063 μg of outer surface protein A (OspA) and OspB, respectively, while 0.0024 μg of OspA and 0.0015 μg of OspB were contained in 1 μg dry weight of BAg. Both BAg and Ag caused blastogenesis in spleen cell cultures from either group of mice, however, BAg-stimulated lymphocytes exhibited significantly greater ($P \leq 0.05$) blastogenesis after 2 or 6 days of culture than lymphocytes stimulated by Ag or medium control. Flow cytometry and antibody capture enzyme-linked immunosorbent assays identified responding lymphocytes as B-cells which secreted polyclonal immunoglobulin M (IgM), but no IgG or IgA. Treatment of BAg and lipopolysaccharide controls with polymyxin B resulted in as much as 20.7 and 54.3 mean percent decrease in blastogenesis, respectively. Fractionation of BAg or Ag by ultracentrifugation prior to culture with spleen cells from naive mice indicated that B-cell blastogenesis was probably associated with spirochetal membranes. The results of this study demonstrate that specific humoral responses are directed towards extracellular membrane blebs which lack the 83, 60 and 41 kDa antigens of intact spirochetes, and that blebs also possess significant nonspecific mitogenic activity for murine B-cells. This activity was not due entirely to typical lipopolysaccharide or OspA and OspB lipoproteins. In order to investigate the mitogenic potential of OspC, another spirochetal surface protein, a 24 kDa protein was first isolated by two-dimensional gel electrophoresis, identified as OspC by immunoblots with rabbit anti-OspC antiserum, and used in lymphocyte proliferation assays which included spleen cells from naive mice. Similarly, purified flagellin from *B. burgdorferi* served as a negative mitogenic control. Mitogenic responses to OspC were significantly greater ($P \leq 0.01$) than flagellin at identical concentrations. Flow cytometry identified responding lymphocytes as B cells. These results indicate that like OspA and OspB, OspC is mitogenic for murine cells and sample processing did not contribute to this effect. Laboratory members have mapped the location of the *ospC* gene, determined its transcriptional start site(s), and compared the expression of the gene among isolates of Lyme disease spirochetes. The results demonstrate that the *ospC* gene is carried on a 26 kb circular plasmid. The *ospC* gene represents the first *Borrelia* gene to be mapped to a circular plasmid. Transcriptional start site analyses revealed that the control region of the gene is similar among isolates of Lyme disease spirochetes and that the gene may be under the control of multiple promoters. Northern (RNA) blot analysis has also demonstrated that the transcriptional expression of the *ospC* gene varies among isolates. Coumermycin A_1 is an inhibitor of DNA gyrase, an enzyme that catalyzes supercoiling of DNA and is required for bacterial DNA replication. Laboratory members have investigated the activity of this coumarin antibiotic on *B. burgdorferi*. *B. burgdorferi* was more susceptible than many other eubacteria to coumermycin as well as novobiocin, another coumarin antibiotic. This contrasted with its relative resistance to the DNA gyrase inhibitors nalidixic acid, oxolinic acid, and ciprofloxacin. Coumermycin at 0.2 μg/ml inhibited the growth of *B. burgdorferi* B31 in BSK II medium. A 100-fold lower concentration induced the relaxation of two negatively supercoiled circular plasmids within 2 hours. Plasmid supercoiling was restored within 2 hours of removal of coumermycin. These results suggest that *B. burgdorferi* has a DNA gyrase and that this enzyme's activity is required for growth. Furthermore, structural analogs of coumermycin may be considered as treatments for Lyme disease. (Garon, Whitmire, Marconi, Samuels, Dorward)

The Molecular Pathogenesis Unit is under the direction of Dr. Witold Cieplak, Jr. The recent publication of the X-ray crystal structure of LT, refined to 1.95 Å resolution, has allowed the investigation of the potential involvement of various residues in a predicted NAD-binding cleft. The refined crystal structure has shown that arginine 7, a residue section members previously showed to be involved in enzymatic activity, lies within the predicted NAD-binding site. More recently, section members have altered histidine 44, serine 61, alanine 69, and tryptophan 127 using multiple isoionic or isosteric substitutions. These residues are predicted to lie within or be vicinal to the substructure or cleft presumed to be the NAD-binding site. While virtually all substitutions at these residues are capable of dramatically reducing enzymatic activity, retention of gross conformation, as judged by sensitivity to limited trypsinolysis, is dependent upon the type of substitution introduced. Mutations that have been judged to result in reductions of enzymatic activity but no detectable alterations in overall conformations are being introduced into the gene for the holotoxin to assess their effects on toxin assembly and cytotonic activity. Members of the Section have begun analyses of potential residues involved in the NAD-binding site using photolabeling techniques. These studies have been facilitated by the development of an FPLC purification scheme that results in preparations of recombinant A subunits and analogs of greater than 95% purity. Direct photolabeling with [carbonyl-^{14}C]-NAD and ultraviolet irradiation has been used and occurs at a level of 20-30 mol %. The labeling is saturable, time dependent, and results in the specific inactivation of enzyme activity. Labeled peptides are being isolated and will be subjected to microsequence analysis to determine the actual site of photolabeling; preliminary analyses using proteolytic fragmentation of the labeled A subunit indicates that the vast majority of the label lies in a peptide containing two glutamic acid residues (110 and 112) that are thought to be critical to catalytic activity. In a collaborative effort with Dr. Boyd Haley (University of Kentucky), members of the section are also employing photoactivatable derivatives of NAD (2-azido-NAD) to map the NAD-binding site. To date, effective photolabeling of recombinant LT A subunit has been achieved at low temperature. Interestingly, both direct photolabeling and photolabeling using 2-azido-NAD have revealed that a co-factor, termed ADP-ribosylation factor (ARF), that is required for the activity of LT and CT appears to bind to the toxins at or near the NAD binding site. These findings will have important implications in establishing the precise nature of the interactions between the toxins, ARF, and the target G proteins. Previous studies have shown that *C. jejuni* alters its protein synthetic profile during interaction with epithelial cells in culture. These observations prompted section members to investigate the nature of the proteins that are novelly synthesized or enhance during the processes of binding and internalization. To facilitate characterization of the genes, several expression libraries of *C. jejuni* genomic DNA were created and section members have been able to maintain genomic fragments in a stable fashion through use of a methyl-mismatch repair deficient strain of *E. coli* as the host. Genomic clones have been screened for preferential reactivity using antisera generated against bacteria grown in the presence and absence of epithelial cells. To date, several clones reacting preferentially with antisera prepared against bacteria cultivated with epithelial cells have been identified. One clone and derivatives have been completely sequenced and they appear to encode the homolog of the widespread bacterial DNA-binding protein termed HU. The preferential immunoreactivity of this protein is puzzling since it is normally quite abundant during bacterial growth. It is noteworthy, however, that the HU gene is only the fifth *C. jejuni* gene to be cloned and sequenced, and as such provides additional information regarding the characteristics of promoters and other regulatory elements in this organism. Additional immunoreactive clones have been identified and are in the process of being characterized. (Cieplak, Konkel, Grant)

In June 1992, a new organization unit was established within the Laboratory of Vectors and Pathogens. The Cell Biology Section, under the direction of Dr. Pam Small, was created to investigate the basic cellular phenomena at work during host-cell interaction, to conduct research on the cytoskeletal rearrangements and intracellular trafficking that occurs when microbial agents enter host cells, and to initiate studies on microorganisms that are members of the genus *Shigella* and the genus *Mycobacterium.* Purchase of a state-of-the art laser confocal microscopy work station (including proprietary image analysis software) to be housed in newly renovated laboratory space and in the assembly of a skilled research group serves to broaden and deepen our bacterial pathogenesis efforts. By linking this group with the transmission and scanning electron facilities already in place in the laboratory, section members insure a uniquely modern and complete center for the full range morphological evaluation and interpretation for both the laboratory and the institute. Furthermore, the expansion of research on other important human pathogens such as *Shigella typhimurium* and *Mycobacterium tuberculosis*, will clearly boost the overall capabilities of the laboratory and institute as well. (Small, Waterman, Pascopella)

A major research goal of the Laboratory of Vectors and Pathogens is to characterize in detail those genes and gen products that are important in bacterial pathogenesis. This knowledge will then be exploited for the development of improved diagnostics and safe and effective new generation vaccines. An additional goal is to understand the mechanisms used by arthropod vectors to maintain and deliver virulent human pathogens.

ADMINISTRATIVE

Drs. Willy Burgdorfer and John Munoz worked as scientist emeritus members of the laboratory during the year, providing valuable service and support.

Guest Researchers in the Laboratory of Vectors and Pathogens have included Dr. Stanley Falkow (Stanford University School of Medicine), Dr. Lucy Tompkins (Stanford University Hospital).

Joining the Laboratory as an IRTA Fellow during the year was Dr. Lisa Pascopella (Albert Einstein College of Medicine). Joining the Laboratory as a Visiting Fellow was Dr. Scott Waterman (The university of Adelaide, South Australia). Beginning a one year sabbatical during the year was Dr. Willard Granath (Division of Biological Sciences, University of Montana). Completing a Staff Fellowship was Dr. William Whitmire. Summer ITRA Appointments included Robert Landry, Yvonne Dehner, JoAnn Cloud and Kathleen Mach.

Visitors/Collaborators who spent varying amounts of time interacting with members of the LVP Scientific Staff included:

Name	Affiliation
Dr. Allan C. Steere	New England Medical Center, Tufts University School of Medicine, Tupper Research Institute, Boston, MA
Dr. Kyung Hee Park	Department of Microbiology, College of Medicine, Kon-Kuk University, Choongju, Choongchungbuk-Do, Korea
Dr. Ulrich E. Schaible	Max-Planck-Institut für Immunobiologie, Stubeweg 51, Freiburg, Germany
Dr. Marty Pavelka	Department of Microbiology and Immunology, University Rochester Medical Center, Rochester, NY
Dr. Kathleen McDonough	Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, NY
Dr. Carleen Collins	Department of Microbiology and Immunology, University of Miami Medical School, Miami, FL
Dr. Jeff Ohman	Department of Microbiology and Immunology, UCLA, Los Angeles, CA
Dr. Robert Perry	Department of Microbiology and Immunology, University of Kentucky Medical Center, Lexington, KY
Dr. Mel Simon	Division of Biology, California Institute of Technology, Pasadena, CA
Dr. Ira Schwartz	Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY
Dr. Joan Slonczewski	Department of Biology, Kenyon College, Danvier, OH

Dr. Erol Fikrig	Yale University School of Medicine, Section of Rheumatology, New Haven, CT
Dr. Alan Barbour	Department of Microbiology, University of Texas Health Science Center, San Antonio, TX
Dr. Richard Wyatt	Office of the Director, Office of Intramural Research, Bethesda, MD

HONORS AND AWARDS

Dr. Claude F. Garon

Served as Chairman, State Board of Governors, Center of Excellence in Biotechnology, Montana Science and Technology Alliance

Served as Faculty Affiliate - Division of Biological Sciences, University of Montana

Served on Internal Advisory Committee, Electron Microscopy Facility, University of Montana

Served as Chairman, Montana Governor's Focus Group on Biotechnology, Montana State Department of Commerce

Served as Poster Session Chairman for 6th Annual Lyme Disease Conference, Atlantic City, New Jersey

Served as Judge at 38th Annual Montana Science Fair, University of Montana.

Served as Editorial Board Member, Journal of Clinical Microbiology

Invited Speaker

Department of Microbiology and Immunology, West Virginia University School of Medicine, Morgantown, West Virginia, October 27-30, 1992

6th Annual Lyme Disease Conference, Atlantic City, New Jersey, May 5-9, 1993

Reviewed Manuscripts

Journal of Clinical Microbiology

The Journal of Infectious Diseases

Dr. Tom Schwan

Award of Merit for Excellence in Technology Transfer, Federal Laboratory Consortium for Technology Transfer, 1993

R. R. Parker Memorial Lecturer, 1993

Program Reviewer for Division of Vector-borne Viral Diseases, CID, CDC, Fort Collins, Colorado, January 1993.

Invited Speaker

Speaker, Annual Meeting of the American Society of Tropical Medicine and Hygiene, Seattle, Washington, November 15, 1992.

Invited speaker, workshops and clinics in numerous cities in Austria, Germany, and Switzerland, March 1993.

Invited speaker, U.S. Forest Service, Hamilton, Montana, April 1993.

Invited speaker, Lyme Disease Foundation, Atlantic City, New Jersey, May 5-9, 1993

Invited speaker, Lyme Disease Vaccine Workshop, Bethesda, Maryland, July 2, 1993

Invited speaker, International Northwestern Conference on Diseases in Nature Communicable to Man, Fort Collins, Colorado, August 8, 1993.

Invited speaker, Rocky Mountain Microbiology Society, Coeur d'Alene, Idaho, September 3, 1993

Reviewed Manuscripts

Journal of Medical Entomology
Journal of Wildlife Diseases
Journal of Clinical Microbiology
Insect Molecular Biology
European Journal of Clinical Microbiology and Infectious Diseases
Journal of Infectious Diseases
Infection and Immunity
American Journal of Tropical Medicine and Hygiene
Proceedings of the National Academy of Science
Molecular Pathogenesis

Dr. Witold Cieplak Jr.

Invited Speaker

First Bristol-Myers Squibb Symposium on Infectious Disease Research, San Francisco, California, February 18-24, 1993.

Bethesda, Maryland, site visit, Laboratory of Bacterial Toxins Program review, CBER, FDA, June 14, 1993.

Editorial Board

Infection and Immunity

Dr. David W. Dorward

Invited Speaker

Ross Laboratories, Columbus, Ohio, January 1993.

St. Patrick Hospital, Missoula, Montana, April 1993.

6th Annual Lyme Disease Foundation Convention, Atlantic City, New Jersey, May 1993.

Reviewed Manuscripts

Journal of Clinical Microbiology

Dr. Willy Burgdorfer

Invited Speaker

International Conference on Tick borne Pathogens, St. Paul, Minnesota, September 15-18, 1992

Interscience Conference on Anti-Microbial Agents and Chemotherapy (ICAAC), Anaheim, California, October 12-13, 1992

The Lyme Coalition of Minnesota, Minneapolis, Minnesota, October 22-23, 1992

Methodist Central Hospital, Memphis, Tennessee, March 22-24, 1993

6th Annual Lyme Disease Conference, Atlantic City, New Jersey, and also honored at a special reception entitled "Hats Off to Willy" in recognition of the discoverer of the Lyme disease causative agent, *Borrelia burgdorferi*, May 5-9, 1993

Served as Instructor at 43nd Acarology Summer Program, Ohio State University, Columbus, Ohio, June 1993

Dr. William Whitmire

Invited Speaker

6th Annual Lyme Disease Conference, Atlantic City, New Jersey, May 5-9, 1993

Dr. Pam Small

Invited Speaker

University of Montana, Missoula, Montana, November 13, 1992

World Conference on Tuberculosis, Bethesda, Maryland, November 1992

First Bristol-Myers Squibb Symposium on Infectious Disease Research, San Francisco, California, February 18-24, 1993

Dr. Scott Samuels

Invited Speaker

6th Annual Lyme Disease Conference, Atlantic City, New Jersey, May 5-9, 1993

Dr. Chris Grant

Invited Speaker

First Bristol-Myers Squibb Symposium on Infectious Disease Research, San Francisco, California, February 18-24, 1993

Dr. Richard Marconi

Invited Speaker

Leptospira Research Conference, Chicago, Illinois, November 6-8, 1992

Dr. Michael Konkel

Invited Speaker

First Bristol-Myers Squibb Symposium on Infectious Disease Research, San Francisco, California, February 18-24, 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00480-08 LVP

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	Tom G. Schwan	Sr Staff Fellow	LVP, NIAID
OTHERS:	J. Hinnebusch	IRTA Fellow	LVP, NIAID
	R. H. Karstens	Bio Lab Tech (Micro)	LVP, NIAID
	W. Burgdorfer	Scientist Emeritus	LVP, NIAID

COOPERATING UNITS *(if any)*

Microbial Diseases Laboratory, California Department of Health, Berkeley (Jane Wong); University of Texas Health Science Center (Alan Barbour); CDC, Fort Collins (Ken Gage).

LAB/BRANCH

Laboratory of Vectors and Pathogens

SECTION

Arthropod-borne Diseases

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Objectives of this project are to (1) use molecular and classical approaches to investigate pathogen-arthropod interactions of vector-borne diseases of human importance in the United States, (2) examine basic biological questions concerning the behavior of ticks and fleas that influence their ability to transmit infectious agents, and (3) to develop rapid and definitive diagnostic assays to detect and identify bacterial pathogens in their arthropod vector.

We applied the polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) rickettsial typing system of Regnery et al. to rapidly identify (within 24 h) rickettsial serotypes in naturally infected ticks. Unlike previously described methods, our PCR assays identify serotypes directly from tick tissues without first isolating the organisms. Thirteen of 226 (5.8%) adult ticks (*Dermacentor andersoni*) collected in the Bitter Root Mountains of western Montana were positive by hemolymph tests using Gimenez stain. These 13 ticks were analyzed by the above PCR/RFLP assay using two different primer sets and restriction enzyme digests. The first primer set (*Rp*CS) reportedly amplifies DNA from all *Rickettsia* serotypes tested to date except *R. tsutsugamushi* serotypes. A second primer set (*Rr*190) amplified target sequences from a subset of spotted fever group rickettsiae that includes all SFG serotypes investigated to date, except *R. akari* and *R. australis*. PCR assays performed using the *Rp*CS primer set resulted in amplification of fragments of the predicted size from 9 of the 13 hemolymph test-positive tick samples. Only 4 of the same 13 tick samples were positive in similar PCR assays performed with the spotted fever group-specific (*Rr*190) primer set. These same 4 tick samples also gave positive results for PCR assays using the *Rp*CS primer set and RFLP analyses indicated these ticks were infected with *R. rickettsii* (1 sample) and *R. rhipicephali* (3 samples). Five of the 9 PCR-positive tick samples were positive only in PCR assays performed with the *Rp*CS primer set. RFLP analyses of PCR fragments amplified from tissues of these 5 ticks indicated they all were infected with *R. bellii*. We also examined established laboratory isolates of tick-borne rickettsiae from different regions of North America to determine whether this typing system produces consistent results. We tested multiple isolates of *R. montana* (9 isolates), *R. bellii* (5 isolates), R. *rickettsii* (Hlp-like) (4 isolates), and *R. canada* (2 isolates) and found no variation in PCR/RFLP patterns between members of the same serotypes. However, among the 5 isolates of *R. rhipicephali* tested, two distinctive RFLP patterns were noted for *Rsa*I digests of PCR fragments amplified using primers for the 190 kilodalton antigen gene of *R. rickettsii*. Our results suggest that this PCR/RFLP typing scheme has wide applicability for identifying rickettsial serotypes directly in ticks.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-464-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 AI 00488-07 LVP

PERIOD COVERED
October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ultrastructural Analysis of Antigenic Determinants in Pathogens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claude F. Garon	Chief	LVP, NIAID
OTHERS:	S. F. Hayes	Bio Lab Tech (Micro)	LVP, NIAID
	W. Burgdorfer	Scientist Emeritus	LVP, NIAID
	D. W. Dorward	Sr Staff Fellow	LVP, NIAID
	L. L. Lubke	Biologist	LVP, NIAID
	T. G. Schwan	Sr Staff Fellow	LVP, NIAID
	W. M. Whitmire	Sr Staff Fellow	LVP, NIAID

COOPERATING UNITS *(if any)*
LPVD, RML, NIAID; LMSF, RML, NIAID; LICP, RML, NIAID; Department of Medical Microbiology, Stanford University School of Medicine

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Structural Pathobiology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.6	2.2	2.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Borrelia burgdorferi, the causative agent of Lyme disease, appears during periods of growth to shed membranous material from its surface. Using an antigen capture/detection method developed in the laboratory, this material could be demonstrated on the surface of spirochetes, free in culture medium and in infected animals and ticks. Although the captured antigens were initially assayed by immune electron microscopy, other methods have been used recently to characterize the nature of these bioproducts and to assess their possible role in the pathogenesis of Lyme disease. Researchers in the laboratory have been able to demonstrate that extracellular components of *B. burgdorferi* 1) appear to be present wherever active growth of the organism is taking place and therefore, may be useful as a diagnostic indicator of active infection and/or treatment effectiveness; 2) are involved in the packaging and protection of intact DNA molecules containing a few known and many unknown genes and gene products; 3) appear to specifically interact with immunoglobulin M molecules in a unique fashion, perhaps to escape immune surveillance; and 4) possesses potent, non-specific mitogenic activity which may cause an inappropriate and noneffective stimulation of the immune system triggering autoimmune disease components. Researchers continue to examine these and other bioproducts with the aim of improving the prevention, treatment and diagnosis of Lyme disease.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-830

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00492-07 LVP

PERIOD COVERED
October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Basis for Infection by *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tom G. Schwan	Sr Staff Fellow	LVP, NIAID
OTHERS:	J. Hinnebusch	IRTA Fellow	LVP, NIAID
	R. H. Karstens	Bio Lab Tech (Micro)	LVP, NIAID
	M. E. Schrumpf	Bio Lab Tech (Micro)	LVP, NIAID
	P. A. Rosa	Sr Staff Fellow	LMSF, NIAID

COOPERATING UNITS *(If any)*
Microbial Diseases Laboratory, California Department of Health, Berkeley (Jane Wong); University of California, Berkeley (Robert Lane); Tufts-New England Medical Center, Boston (Mark Klempner).

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Arthropod-borne Diseases

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to (1) use recombinant DNA techniques to express specific antigens of *Borrelia burgdorferi* to improve the serodiagnosis of Lyme disease, (2) characterize at the molecular level, isolates of the Lyme disease spirochete from a wide range of biological and geographical sources, and (3) to use animal models to help understand the pathogenesis of Lyme disease and to examine serological reponses to infection.

During the last year we entered into a collaboration with Korean scientists to examine the potential for human Lyme disease in Korea. Lyme disease spirochetes, *Borrelia burgdorferi* sensu lato, were identified and characterized for the first time from Korea. Four isolates, designated Konkuk-1, Konkuk-2, Kangwon-3, and KM-4 were made from midgut suspensions of three *Ixodes* ticks and heart tissue from one mouse, *Apodemus agrarius*, collected from Chungbuk and Kangwon Provinces. The four Korean isolates and *B. burgdorferi* sensu lato from other geographic areas and biological sources were compared using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for protein profiles, Western immunoblot analysis for reactivities with monoclonal and polyclonal antibodies, and agarose gel electrophoresis for plasmid profiles. Two typing schemes using the polymerase chain reaction (PCR) identified three of the isolates as members of group VS461 and one, Kangwon-3, as *B. garinii*. These results demonstrate the potential for human Lyme disease to occur in some provinces of Korea.

Previous studies have demonstrated that the urinary bladder is a consistent source for isolating the Lyme disease spirochete, *Borrelia burgdorferi*, from both experimentally infected and naturally exposed rodents. We examined histopathological changes in the urinary bladder of different types of rodents experimentally infected with Lyme spirochetes, including BALB/c mice (*Mus musculus*), nude mice (*M. musculus*), white-footed mice (*Peromyscus leucopus*), and grasshopper mice (*Onychomys leucogaster*). Animals were inoculated intraperitoneally, subcutaneously, or intranasally with low-passaged spirochetes, high-passaged spirochetes, or phosphate-buffered saline. At various times following inoculation, animals were sacrificed and approximately one-half of each urinary bladder and kidney were cultured separately in BSK-II medium while the other half of each organ was prepared for histological examination. Spirochetes were cultured from the urinary bladder of all 35 mice inoculated with low-passaged spirochetes while we were unable to isolate spirochetes from any kidneys of the same mice. The pathological changes observed most frequently in the urinary bladder of the infected mice were the presence of lymphoid aggregates, vascular changes including an increase in the number of vessels and thickening of the vessel walls, and perivascular infiltrates. Our results demonstrate that nearly all individuals (93%) of the four types of mice examined had a cystitis associated with spirochetal infection.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-284-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00552-05 LVP

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathobiology of Bacterial Toxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Cieplak, Jr.	Sr Staff Fellow	LVP, NIAID
OTHERS:	C. C. R. Grant	Visiting Associate	LVP, NIAID
	R. J. Messer	Microbiologist	LVP, NIAID

COOPERATING UNITS *(if any)*

Boyd E. Haley (University of Kentucky); Paul Offit (Childrens Hospital, Philadelphia, PA); Richard Kahn (NCI, NIH)

LAB/BRANCH

Laboratory of Vectors and Pathogens

SECTION

Molecular Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Efforts to elucidate the particular regions and residues of *Escherichia coli* heat-labile enterotoxin involved in toxic and enzymatic activity have continued. Based on the recently published crystal structure that predicts the position of an NAD-binding site in the A subunit, we have subjected a number of residues to site directed mutagenic alteration. Analyses of the mutant analogs has revealed that in most cases alterations which abrogate ADP-ribosyltransferase activity also impart conformational changes that are detectable by tryptic fragmentation patterns. However, conservative substitution at tryptophan 170 by tyrosine yields a molecule with reduced activity and apparent wild-type conformation. This mutation, in conjunction with other previously described substitutions may be a suitable target for the generation of, multiply substituted mutant proteins that are devoid of toxic/enzymatic activity and that retain native conformation. We are continuing to investigate various amino acid substitutions at various target in the hope of generating mutants with the appropriate phenotypic characteristics (i.e. no enzymatic activity and no gross conformational alterations).

Additional studies have been conducted to evaluate the effect of post-translational modifications on the enzymatic activity of the A subunit of LT. These studies have revealed that limited proteolytic cleavage is not necessary for the expression of enzymatic activity and that multiple auto-or self ADP-ribosylation does not detectably change the specific enzymatic activity of the toxin. These findings contrast the results of studies conducted by other groups using cholera toxin.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 AI-00553-05 LVP

PERIOD COVERED
October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology and Immunology of Pathogenic *Campylobacter* spp.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Cieplak, Jr.	Senior Staff Fellow	LVP, NIAID
OTHERS:	M. E. Konkel	IRTA Fellow	LVP, NIAID
	D. J. Meade	Microbiologist	LVP, NIAID

COOPERATING UNITS *(if any)*
Stanford University Medical School (Lucy Tompkins); University of Arizona College of Veterinary Sciences (Lynn Joens)

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Molecular Pathogenesis Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	**PROFESSIONAL:**	**OTHER:**
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

We have continued to focus our attention on the interaction of *Campylobacter jejuni* with human epithelial cells particularly with respect to altered protein synthesis that occurs during the binding and internalization of *C. jejuni.* Antisera prepared against organisms grown in the presence of epithelial cells are capable of inhibiting translocation and have been used to screen recombinant expression libraries of *C. jejuni* genomic DNA. Several clones have been identified that produce proteins that are preferentially recognized by antisera produced against bacteria cultured with epithelial cells. One clone has been completely sequenced and appears to encode the homolog of the bacterial DNA-binding protein HU. Additional studies have characterized *in vitro* phenotypic passage variants of C. jejuni. A high passage variant of a clinical isolate termed M96 that is defective for internalization within epithelial cells has also been obtained. However, this variant is fully competent at translocating across monolayers of polarized epithelial cells. At the molecular level, the strain differs only from the parent in terms of the structure of lipopolysaccharide (LPS). This finding may indicate a specific role for LPS in internalization and further suggests that translocation across polarized cells occurs by a different mechanism than that associated with internalization or invasion of conventionally cultured cells.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-884-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00554-05 LVP

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Characterization of Microbial Genes and Nucleic Acid Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claude F. Garon	Chief	LVP, NIAID
OTHERS:	R. T. Marconi	IRTA Fellow	LVP, NIAID
	D. W. Dorward	Sr Staff Fellow	LVP, NIAID
	L. L. Lubke	Biologist	LVP, NIAID
	D. S. Samuels	IRTA Fellow	LVP, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Vectors and Pathogens

SECTION

Structural Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.3	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Lyme borreliosis is now the most common arthropod-borne disease in the United States. *Borrelia burgdorferi*, the causative agent, has been isolated from humans, mammals, birds and arthropods and is cultivable in the laboratory. Careful structural analysis of the DNA content of several early passage isolates has revealed, in addition to a 1,000 kb linear genome, a unique mixture of terminally cross-linked linear and covalently-closed, circular DNA molecules ranging in size from 2 to 50 kilobases in length. Often DNA patterns appear to differ among isolates and to vary during laboratory passage.

We have determined and compared partial 16S rRNA sequences from 23 Lyme disease spirochete isolates and aligned these with 8 sequences previously presented. The 16S rRNA signature nucleotide compositions were defined for each isolate and compared with the genomic species signature nucleotide sets previously established. Several positions were found to be variable in identity, particularly in European isolates. To identify positions truly indicative of the genomic species classification which could serve as targets for polymerase chain reaction (PCR) speciation primers, 16S rRNA based phylogenetic analyses were conducted. Based upon the identified signature nucleotides, we designed PCR primer sets which 1) amplify all spirochete species associated with Lyme disease and 2) differentiate between these species. The primer sets were tested and found to be sensitive and specific. All Lyme disease isolates tested were amplification positive. These primers should allow for the rapid speciation of isolates and for improved clinical diagnosis of Lyme disease as well.

The objective of this project, therefore, is to define the genetic capacity of *B. burgdorferi* and other vector-borne pathogens in sufficient detail to begin the process of mapping those genes and gene products which may be important in pathogenesis.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-884-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00670-01 LVP

PERIOD COVERED
December 1, 1992, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Genetic and Physiological Basis of Acid Resistance in Enteric Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.L.C. Small	Sr Staff Fellow	LVP, NIAID
OTHERS:	Scott Waterman	Visiting Fellow	LVP, NIAID
	Diane Welty	Microbiologist	LVP, NIAID

COOPERATING UNITS *(if any)*
Dr. Joan Slonczewski, Department of Biology, Kenyon College, Danvier, Ohio

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Cell Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.74	.58	.16

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Shigella species are unique among enteric pathogens in the low infective dose required for disease. Whereas over 10^6 *Vibrio cholera* or 10^5 *Salmonella* species are required to produce disease, 200 or less shigellae are able to do so. *Shigella* species are able to survive a pH of less than 3.0 for several hours; the ability to survive low pH is an important determinant of infective dose.

The objectives of this project are 1) to characterize the genetic and physiologic basis of acid resistance in *Shigella* species and 2) to utilize this information in the construction of better oral vaccine strains. We have isolated a clone from a library of *S. flexneri* DNA which confers acid resistance on an acid sensitive *Escherichia coli*. Deletion analysis and complementation studies identified the acid resistance determinant as a homologue of the *E. coli* stationary phase sigma factor, *rpo*^{s}. We have sequenced the gene and found over 90% DNA identity between the *Shigella* and *E. coli* open reading frame for *rpo*^{s}. However, there are significant differences between two in the upstream regulatory portion of the gene. Measurement of internal pH in the face of falling external pH show that wild type *S. flexneri* is able to maintain a significantly higher internal pH when exposed to an external pH below 4.0 than is a *S. flexneri rpo*^{s}:Tn10. This suggests that *rpo*^{s} may regulate the expression of proteins important for transport of ions across the cytoplasmic membrane. We are in the process of identifying *rpo*^{s} regulated genes required for survival at low pH. Twenty-nine acid sensitive TnPhoA mutants have been isolated and are being characterized. An analysis of acid survival in *S. flexneri* will provide a basis for designing better oral vaccine strains since delivery of antigens to the intestines will be enhanced by better survival of strains through the stomach.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 A1 00671-01 LVP

PERIOD COVERED

January 4, 1993, to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular and Cell Biology of Mycobacterial Species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Pam L. C. Small	Sr Staff Fellow	LVP, NIAID
OTHERS:	Diane Welty	Microbiologist	LVP, NIAID
	Lisa Pascopella	IRTA Fellow	LVP, NIAID
	Stanley Falkow	Collaborating Scientist	Stanford University

COOPERATING UNITS *(If any)*

Eric Langhoff, Dana-Farber Cancer Institute, New York, NY; Lalita Ramarkrishnan, Stanford University, Stanford, CA

LAB/BRANCH

Laboratory of Vectors and Pathogens

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.96	.91	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are 1) to characterize both the cellular and bacterial components of mycobacterial entry into and survival within host cells and 2) to characterize the state of "dormant" Mycobacteria and identify bacterial determinants necessary for long-time survival in the host. We have begun our investigations by exploring the usefulness of *M. marinum* and *M. ulcerans*, as a model for *M. tuberculosis*. These mycobacterial species are taxonomically a part of the *M. tuberculosis* complex. Initial work has focussed on understanding the relationship between *M. marinum* and host cells. We have found that *M. marinum* have the ability to adhere to, enter and replicate within macrophage, epithelial and fibroblast cell lines. Electron micrographs demonstrate that early in infection intracellular organisms are found within membrane bound compartments. Long-term infection of fibroblast and macrophage cell lines results in detachment of cells from the monolayer. In contrast to *M. marinum*, *M. ulcerans* neither replicates nor adheres to host cells. However, both live bacteria as well as sterile filtrates from *M. ulcerans* cultures are cytotoxic for host cells. We have found that cytotoxicity is a characteristic of stationary phase cultures only. Further investigation will be needed to define the intracellular compartment within which mycobacteria replicate. We plan to identify genetic determinants responsible for cell entry, intracellular growth and cytotoxicity. With the completion of a P-3 facility required for working with virulent *Mycobacterial tuberculosis*, we will extend this work by looking at the interaction of *M. tuberculosis* with host cells.

All *M. marinum* examined so far contain a 3 kb and 5 kb plasmid. These are the only plasmids identified from bacteria in the *M. tuberculosis* complex. We plan to explore the use of these plasmids for developing a genetic system for use in *M. tuberculosis*.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

ROCKY MOUNTAIN OPERATIONS BRANCH
ROCKY MOUNTAIN LABORATORIES
Hamilton, Montana

ANNUAL REPORT FY 1993

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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1992 to September 30, 1993

Introduction

The Operations Branch provides support in the following areas to facilitate the research being conducted by the four laboratories at RML: library services, grounds care, janitorial service, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography/graphic arts services, animal rearing and care, motor pool, operation of the power plant, and full maintenance and minor laboratory renovations.

Some major projects have been accomplished in refurbishing/improving physical facilities during the past year. The 100 foot masonry stack for the heating plant was razed and replaced with a new steel, refractory lined stack. Concurrent with this project, the asbestos in the boiler room was removed and the catwalks in the boiler room were rebuilt to meet OSHA standards. Space was renovated in building 13 to provide more adequate men's and women's locker rooms and a new lunch room was constructed. Space in building 13 was also renovated to provide a non-human primate surgery room. Work was initiated to convert HD-1 into a biohazard class 3 facility which will be utilized for TB research. Work was also initiated to install a fire alarm system, evacuation signage, emergency lighting and other renovations to correct fire safety deficiencies in buildings 1-7 and 10. Old, unrequired facilities were razed which included dog runs and mink sheds in the old MK-1 and MK-2 compounds.

The maintenance section completed a number of renovation and repair projects for the laboratories and animal unit. Space was renovated to create a new post-doc office for LMSF and lab space for LICP was renovated. Ventilation and lighting was improved in ARMCO 1 & 2 and a new small animal quarantine room was constructed in the east end of ARMCO 2. Concrete slabs and walkways were installed in the lower mink yard and around ARMCO 1 & 2.

Several projects were completed to upgrade facilities in the animal care section. Work was completed on the security contract to detect unauthorized intrusions into the buildings. New stainless steel caging was installed in the lower mink yard; all experimental mink are now housed in these cages. As mentioned above, a small animal quarantine room was constructed for holding/testing of small animals before allowing them to be introduced into building 12 or 13 if their health status is uncertain. Three flexible film isolators were installed in building 12 to provide a pathogen free environment in which to rear foundation stock of our unique (one-of-a-kind) strains of RML mice.

Operations Branch personnel assisted in providing host support for the Board of Scientific Counselors meeting and the third annual lab retreat of LPVD and Irv Weissman's group from Stanford University.

General Overview of the Responsibilities of Operations Branch

The Chief of the Branch is responsible for labor management work and administrating the technical aspects of the contracts for Security, Janitorial, operation of the Boiler Room and the Glassware Unit with the respective private contractors. Security is provided by a guard on duty every night and all day on weekends and holidays. Janitorial services are provided in five laboratory buildings daily except weekends and holidays. Boiler Room operation provides heat, steam, compressed air, vacuum, and emergency power to the entire laboratory complex. Glassware is cleaned and sterilized in the Glassware Unit for reuse in the laboratories.

The Maintenance Section provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning and refrigeration, including ultra-low temperature boxes. With exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 9 vehicles is maintained. Grounds care including snow removal is provided.

Most of the biological media used in research laboratories is prepared in a media kitchen by a technician.

The Graphic Arts Department provides full professional services to the laboratory staffs with the exception of medical artistry.

The library provides a full range of services for the RML staff, i.e., selections and acquisitions, cataloging, circulation, inter-library loans, reference and bibliographic services, computerized data base searches, and preparing periodicals for binding.

The Animal Care Section raises 1 strain of mink, 21 strains of mice, 6 strains of hamsters, a colony of grasshopper mice, a colony of peromyscus and a colony of microtus. Approximately 45,000 research animals are reared annually from an average pool of 3,000 breeding stock. An additional 6,000 - 7,000 animals are purchased annually from outside sources, including mink, rabbits, rats, mice, hamsters and guinea pigs. The most recent survey indicates an average daily holding of 14,000 small animals. An experimental colony of 67 cynomolgus macaques, as well as 2 horses, 2 cattle and 2 sheep are also maintained in the animal facilities.

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